PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/19, C07K 14/52, C12N 5/10, C12Q 1/68, C07K 16/24, A61K 38/19, 37/70, A01K 67/027, G01N 33/68

A1

(11) International Publication Number: WO 00/20593

(43) International Publication Date: 13 April 2000 (13.04.00)

(21) International Application Number: PCT/US99/22678

(22) International Filing Date: 30 September 1999 (30.09.99)

(30) Priority Data:

60/102,883	2 October 1998 (02.10.98)	US
60/110,405	1 December 1998 (01.12.98)	US
60/138,910	11 June 1999 (11.06.99)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Applications

US	60/102,883 (CIP)
Filed on	2 October 1998 (02.10.98)
US	60/110,405 (CIP)
Filed on	1 December 1998 (01.12.98)
US	60/138,910 (CIP)
Filed on	11 June 1999 (11.06.99)

(71) Applicant *(for all designated States except US)*: ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: GLASEBROOK, Andrew, L. [US/US]; 11410 Valley Meadow Drive, Zionsville, IN 46077 (US). SU, Eric, W. [CN/US]; 13447 Dunes Drive, Carmel, IN 46032 (US). WEI, Jian-Jun [CN/US]; 25 Cinder Road, Oaktree Village 1A, Edison, NJ 08820 (US). LIU, Ling [CA/US]; 10740 Putnam Place, Carmel, IN 46032 (US).

(74) Agents: BENT, Stephen, A. et al.; Foley & Lardner, Suite 500, 3000 K Street, N.W., Washington, DC 20007-5109 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: IL-17 HOMOLOG NUCLEIC ACIDS, POLYPEPTIDES, VECTORS, HOST CELLS, METHODS AND USES THEREOF

(57) Abstract

The present invention relates to at least one novel IL-17 homolog polypeptide, including isolated nucleic acids that encode at least one IL-17 homolog polypeptide, IL-17 homolog polypeptides, vectors, host cells, transgenics, chimerics, and methods of making and using thereof same, as well as IL-17-homolog-specific antibodies and methods.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

IL-17 HOMOLOG NUCLEIC ACIDS, POLYPEPTIDES, VECTORS, HOST CELLS, METHODS AND USES THEREOF

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to compounds and compositions comprising novel human interleukin-17 (IL-17) homolog polypeptides and nucleic acids. More specifically, recombinant or isolated nucleic acid molecules are provided encoding human IL-17 homolog polypeptides as well as vectors, host cells, antibodies and methods for producing and using different aspects of the invention.

RELATED ART

Considerable effort has been exerted to identify T cell-derived molecules that are unique to the activation state of a T cell or specific to T cell functions (Brunet et al., Nature 322:268 (1986); Bleackley et al., Immunol. Rev. 103:5 (1988); Rouvier et al., J. Immunol. 150:5445 (1993); and Lancki et al., J. Immunol. 146:3242 (1991)). The recent discovery of such molecules, coupled with their sequence comparisons to known gene families and superfamilies, has contributed to an overall understanding of the processes involved in, and the consequences of, T cell activation. One such recently discovered molecule is cytotoxic T-lymphocyte-associated antigen-8 (CTLA-8), a novel 20 kDa secreted glycoprotein that appears to be part of a unique ligand-receptor system that regulates the production of various cytokines (Yao et al., J. Immunol. 155:5483 (1995) and Broxmeyer, J. Exp. Med. 183:2411 (1996)). Because CTLA-8 is a secreted factor and because its biological activities classify it as a cytokine, the designation IL-17 has more recently been suggested for this factor.

cDNA clones encoding IL-17 have been isolated from activated rat, mouse and human T cells. An IL-17 specific mouse cell surface receptor (IL-17 R) has recently been cloned. While the expression of IL-17 mRNA is restricted to activated T cells, the expression of mIL-17 R mRNA has been detected in virtually all cells and tissues tested. IL-17 exhibits multiple biological activities on a variety of cells including: the induction of IL-6 and IL-8 production in fibroblasts; the enhancement of surface expression of ICAM-1 in fibroblasts; activation of NF-kB and costimulation of T cell proliferation. It is also a potent stimulator of bone resorption. The non-glycosylated, Escherichia coli-expressed recombinant human IL-17 (rhIL-17) has been shown to be active on human as well as mouse cell lines.

Interleukin 17 was originally cloned from a T cell hybridoma produced by fusion of a mouse cytotoxic T cell clone and a rat T cell lymphoma (Rouvier et al., J. Immunol. 150:5445 (1993)). The sequence of murine IL-17 (mIL-17) was first identified (as CTLA-8) from a murine cDNA sequence obtained by subtractive hybridization of an activated T-cell hybridoma (Rouvier et al., J. Immunol. 150:5445 (1993)). The corresponding putative 150 amino acid sequence predicted that mIL-17 could encode a low molecular weight protein with a signal peptide. The mIL-17 cDNA sequence contains an AU-rich repeat in the 3'-untranslated region of the mRNA such as those previously found in the mRNA of various cytokines, growth factors, and oncogenes.

Recombinant mIL-17 exhibits activities on a variety of cell types including activation of NF-kappaB, which is known to regulate a number of gene products involved in cell activation and growth control (Yao et al., Immunity 3:811-821 (1995)); induction of IL-6 (Yao et al., Immunity 3:811-

821 (1995)) and IL-8 (Yao et al., J. Immunol. 155:5483-5486 (1995)) secretion; ICAM-1 expression in fibroblasts (Yao et al., J. Immunol. 155:5483-5486 (1995)); and costimulation (Fossiez et al., J. Exp. Med. 183:2593-2603 (1996)) of T-cell proliferation induced by sub-optimal stimulation with PHA. The murine IL-17 receptor (mIL-17R) was identified when proteins were screened for binding to an HVS13-Fc fusion protein. A library of cDNA was expressed and screened for binding proteins, leading to two isolates that bound mIL-17. Soluble mIL-17R inhibited T-cell proliferation and IL-2 production induced by conA, PHA or anti T-cell receptor antibody, suggesting a key role for endogenously produced mIL-17 in T-cell activation.

The human equivalent of mIL-17 was cloned based on its homology to the rodent sequence and to an open reading frame of Herpesvirus saimiri and was designated IL-17 (Yao et al., J. Immunol. 155:5483 (1995) and Fossiez et al., J. Exp. Med. 183:2593 (1996)). In addition to the rat and human factors, mouse IL-17 has also been cloned (Yao et al., Immunity 3:811 (1995) and Kennedy et al., J. Interferon Cytokine Res. 16:611 (1996)).

Human IL-17 (hIL-17) is a variably glycosylated, 20-30 kDa homodimeric polypeptide reportedly secreted by CD4+ activated memory (CD45+RO+) T cells (Yao et al., J. Immunol. 155:5483 (1995) and Fossiez et al., J. Exp. Med. 183:2593 (1996)). The human IL-17 gene codes for a 155 amino acid (aa) residue protein that consists of a 19 aa residue signal sequence and a 136 aa residue mature segment. Within the mature polypeptide, there are six cysteine residues and one potential N-linked glycosylation site (Yao et al., J. Immunol. 155:5483 (1995) and Fossiez et al., J. Exp. Med. 183:2593 (1996)). Dimerization is achieved through

intermolecular cysteine bonds (Yao et al., J. Immunol. 155:5483 (1995)).

Rat IL-17 is a 150 aa residue polypeptide with a signal sequence of 13 aa residues, and a mature polypeptide length of 137 aa residues (Yao et al., J. Immunol. 155:5483 (1995) and Fossiez et al., J. Exp. Med. 183:2593 (1996)).

Mouse IL-17 is a 158 aa residue polypeptide with a predicted signal sequence of 21 aa residues and a mature polypeptide length of 137 aa residues (Kennedy et al., J. Interferon Cytokine Res. 16:611 (1996)).
Mouse and rat IL-17 show 87.3% sequence identity to each other at the amino acid level (Kennedy et al., J. Interferon Cytokine Res. 16:611 (1996)). Human IL-17 shows amino acid sequence identity of 62.5% and 58% to the mouse and rat sequences, respectively (Kennedy et al., J. Interferon Cytokine Res. 16:611 (1996)). High levels of hIL-17 were induced from primary peripheral blood CD4+ T-cells upon stimulation, but not detected in unstimulated peripheral blood T-cells, peripheral blood cells, an EBV-transformed B-cell line, or a T-cell leukemia line. Furthermore, no detectable signals were observed in several other tissues including lung, liver, heart, kidney and spleen. When expressed in CV-1/EBNA cells, recombinant hIL-17 was secreted in both glycosylated and nonglycosylated forms. When the immunoprecipitates with murine anti-human IL-17 antibody were analyzed under nonreduced conditions, hIL-17 was present mainly as 30 and 38kDa proteins, suggesting that hIL-17 dimerizes through cysteine bonds.

Human IL-17 has the capacity to induce the production of other cytokines from stromal cell elements, such as fibroblasts, endothelial cells and epithelial cells. Thus, it has been determined that hIL-17 can induce the release of IL-6, IL-8, G-CSF, and PGE2 (Fossiez et al., J. Exp. Med.

183:2593-2603 (1996)) and can also enhance the surface expression of ICAM-1. TNF-alpha and IFN-gamma were found to have additive effects on the hIL-17-induced secretion of IL-6; while neither hIL-17 nor TNF-alpha induced release of GM-CSF, the combination of hIL-17 plus TNF-alpha was effective in this event. On the other hand, the expression of MHC-class I, MHC-class II, and LFA-1 molecules was not significantly affected. hIL-17 had no major effect on the proliferation, cytokine secretion (IFN-gamma, IL-4, IL-6, IL-10), phenotype (CD3, CD4, CD8), or cytotoxicity of total PBMC or purified CD4+ and CD8+ T-cells, regardless of whether these cells had been activated with PHA, tetanus toxoid, or IL-2. hIL-17 did not affect the proliferation and Ig production of normal tonsillar B-cells activated either through antigen receptor or through CD40. hIL-17 did not induce proliferation or differentiation of cord blood CD34+ hematopoietic progenitors cultured with or without GM-CSF.

Studies now suggest that IL-17 may be a major vehicle by which T cells communicate with the hematopoietic system. In particular, fibroblasts, when cultured in the presence of IL-17, are able to sustain CD34+ hematopoietic progenitor cells and direct their maturation towards neutrophils (Fossiez et al., J. Exp. Med. 183:2593 (1996)). The exact pathway(s) involving IL-17 is not clear. However, IL-17 has been demonstrated to induce IL-6, IL-8 and G-CSF production by fibroblasts (and endothelium), and these three cytokines are known to impact hematopoiesis (Broxmeyer, J. Exp. Med. 183:2411 (1996) and Fossiez et al., J. Exp. Med. 183:2593 (1996)). IL-6, for instance, induces hematopoietic progenitor cells to form granulocyte/macrophage colonies (Ikebuchi et al., Proc. Natl. Acad. Sci. USA 84:9035 (1987)), while G-CSF, both in vitro (Berliner et al., Blood 85:799 (1995)) and in vivo (Roberts et al., Exp. Hematol.

22:1156 (1994)), accelerates the formation of neutrophils. IL-8, in contrast, seems to downmodulate the effects of myelopoietic-promoting cytokines, suggesting that IL-17 may ultimately be found to have the ability to fine-tune or impact all general phases of a hematopoietic response (Broxmeyer, J. Exp. Med. 183:2411 (1996) and Broxmeyer et al., J. Immunol. 150:3448 (1993)). Thus, it appears that IL-17 can now be considered the newest T-cell-derived hematopoietic cytokine, joining IL-3 (Dilloo et al., Exp. Hematol. 24:537 (1996) and Huhn et al., Exp. Hematol. 24:839 (1996)), IL-4 (Rennick et al. IL-4: Structure and Function. H. Spits Ed., CRC Press, Boca Raton, p. 151 (1992)), IL-5 (Takatsu et al., Adv. Immunol. 57:145 (1994)) and GM-CSF (Kruger et al., Immunology 88:49 (1996) and Hill et al., J. Leukoc. Biol. 58:634 (1995)) as either regulators or co-regulators of hematopoiesis (Fossiez et al., J. Exp. Med. 183:2593 (1996)).

It is not yet clear how widespread the expression of IL-17 is. Early reports implicated T-lymphocytes as the major source of IL-17 (Yao et al., J. Immunol. 155:5483 (1995); Broxmeyer, J. Exp. Med. 183:2411 (1996); and Fossiez et al., J. Exp. Med. 183:2593 (1996)), particularly activated memory CD4+ T cells (Fossiez et al., J. Exp. Med. 183:2593 (1996)). More recent studies have suggested a different and more restricted expression pattern in mice (Kennedy et al., J. Interferon Cytokine Res. 16:611 (1996)). Expression of mouse IL-17 by various activated T cells could only be detected using PCR analysis. Using Northern analysis only a subset of T cellswere found to express IL-17 at physiologically significant levels (Kennedy et al., J. Interferon Cytokine Res. 16:611 (1996)). These cells are among the first to be activated during immune responses and are capable of producing large amounts of cytokines

characteristic of Th2 type responses (IL-4, IL-5, IL-10, and IL-13) as well as factors usually associated with CD8+ T cells (IFN-g, TNF-b, RANTES, granzyme B, and Fas ligand) (Kennedy et al., J. Interferon Cytokine Res. 16:611 (1996)). Although these results suggest that IL-17 is not expressed by a wide variety of cell types, it is possible that other cells may express higher levels of IL-17 when appropriate activation conditions are found. This work also suggests that IL-17 may play a role in the early stages of immune responses.

The observations that IL-17 is produced by activated CD4+ T cells, activates T cells, and acts on many cells and tissues that ubiquitously express the IL-17 R suggests it may function as a proinflammatory protein.

SUMMARY OF THE INVENTION

The present invention provides isolated nucleic acids and encoded novel IL-17 homolog polypeptides, including fragments and specified variants, as well as IL-17 homolog polypeptide compositions, vectors, host cells, antibodies, and methods, as described and enabled herein.

Nucleic Acid Molecules

The present invention provides, in one aspect, isolated nucleic acid molecules comprising polynucleotides encoding IL-17 homolog polypeptides and fragments thereof.

The present invention further provides an isolated nucleic acid that hybridizes under stringent conditions to SEQ ID NO:1 or the complement thereof. The present invention further provides an isolated nucleic acid that hybridizes under stringent conditions to nucleotides 55-591 of SEQ ID NO:1 or the complement thereof. The present invention further provides an isolated nucleic acid having at least 80% sequence identity to SEQ ID NO:1 and a nucleic

acid having at least 80% sequence identity to nucleotides 55-591 of SEQ ID NO:1.

The present invention also provides an isolated nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:2 and an isolated nucleic acid sequence encoding amino acids 19-197 of SEQ ID NO:2.

The present invention further provides an isolated nucleic acid sequence corresponding or complementary to at least 90-100% of the contiguous nucleotides of SEQ ID NO:1.

The isolated nucleic acids described herein preferably have IL-17 homolog polypeptide activity as described herein.

The present invention also provides methods of making or using such nucleic acids, vectors and/or host cells, such as but not limited to, using them for the production of nucleic acid encoding IL-17 homologs and/or IL-17 homolog polypeptides by known recombinant, synthetic and/or purification techniques, based on the teaching and guidance presented herein in combination with what is known in the art.

The present invention also provides a vector comprising an isolated nucleic acid encoding an IL-17 homolog as described herein.

The present invention also provides a vector as described herein, wherein the vector is selected from a linear or circular, single or double stranded, DNA, RNA, or combination thereof, nucleic acid vector.

The present invention also provides an isolated nucleic acid probe as described herein, wherein the nucleic acid comprises, for example, a polynucleotide of preferably at least 10 contiguous nucleotides of SEQ ID NO:1.

Polypeptides

The present invention also provides an isolated IL-17 homolog polypeptide as described herein, comprising the amino acid sequence of SEQ ID NO:2.

The present invention further provides an isolated IL-17 homolog polypeptide comprising amino acids 19-197 of SEQ ID NO:2.

The present invention further provides an isolated IL-17 homolog polypeptide comprising a polypeptide having at least 80% sequence identity to SEQ ID NO:2 or at least 80% sequence identity to amino acids 19-197 of SEQ ID NO:2.

The present invention also provides an isolated IL-17 homolog polypeptide comprising a variant of SEQ ID NO:2 or a fragment of SEQ ID NO:2, where the variant or fragment preferably has IL-17 homolog polypeptide activity as described herein.

The present invention further includes an isolated IL-17 homolog polypeptide comprising at least 90-100% of the contiguous amino acids of the amino acid sequence of SEQ ID NO:2.

The present invention also provides a host cell, comprising an isolated nucleic acid encoding an IL-17 homolog as described herein.

The present invention also provides a method for constructing a recombinant host cell that expresses an IL-17 homolog polypeptide, comprising introducing into the host cell a nucleic acid encoding an IL-17 homolog in replicatable form as described herein to provide the recombinant host cell. The present invention also provides a recombinant host cell provided by methods as described herein.

The present invention also provides a method for expressing an IL-17 homolog polypeptide in a recombinant

host cell, comprising culturing a recombinant host cell as described herein under conditions wherein the IL-17 homolog polypeptide is expressed in detectable or recoverable amounts.

The present invention also provides at least one isolated IL-17 homolog polypeptide produced by a recombinant, synthetic and/or purification method as described herein and/or as known in the art.

Compositions and pharmaceutical compositions comprising at least one IL-17 homolog polypeptide made according to such processes are also provided.

The present invention also provides isolated IL-17 homolog polypeptides as described herein, wherein the polypeptides have at least one activity selected from induction of cytotoxic T cells, induction of lymphokine-activated killer cell proliferation, or B or T cell stimulation (Lowenthal et al, Nature, 315:669 (1985); Smith, Science, 240:1169 (1988); Biron et al., J. Exp. Med., 171:173 (1990); Spagnoli et al., Cell. Immunol., 146:391 (1993)).

The present invention further provides IL-17 homolog polypeptides that enhance, *e.g.*, stimulate, expression and/or secretion of IL-6, IL-8, and/or ICAM-1 in animal cells. Such activity can be assayed via methods known to the skilled artisan and via methods described herein.

An IL-17 homolog polypeptide can thus be screened for an activity according to known methods.

The present invention also provides a composition comprising an isolated nucleic acid encoding an IL-17 homolog polypeptide and/or an isolated IL-17 homolog polypeptide and a carrier or diluent. The carrier or

diluent can optionally be pharmaceutically acceptable, according to known methods.

Methods of Using IL-17 Homolog Polypeptides

The present invention also provides a method for modulating, *e.g.*, stimulating, hematopoiesis, including erythropoiesis (production of red blood cells), leukopoiesis (production of white blood cells) and/or thrombocytopoiesis (production of platelets), said method comprising: administering a therapeutically-effective amount of a pharmaceutical composition comprising at least one IL-17 homolog polypeptide to a cell, tissue, organ, animal or patient in need of such therapy.

Furthermore, the present invention provides IL-17 homolog polypeptides and compositions that modulate, *e.g.*, stimulate intracellular signaling pathways dependent on at least one of hematopoietic, erythropoietic, leukopoietic or thrombopoietic related functions. Such polypeptides and compositions stimulate or inhibit T cell activation and/or proliferation and, thereby, have therapeutic utility for treating infections caused by retroviruses including but not limited to, HIV. Furthermore, polypeptides and compositions can stimulate or inhibit T cell activation and/or proliferation and, thereby, have therapeutic utility for treating various autoimmune diseases including, but not limited to, rheumatoid arthritis, lupus, graft versus host, host versus graft, insulin-dependent diabetes, autoimmune encephlomyelitis, and multiple sclerosis.

Antibodies and Methods of Use Thereof

The present invention also provides an IL-17 homolog antibody or fragment, comprising a polyclonal and/or monoclonal antibody or fragment that specifically binds at least one epitope specific to an isolated IL-17 homolog polypeptide as described herein.

The present invention also provides a method for providing an IL-17 antibody or fragment, comprising generating the antibody or fragment that binds at least one epitope that is specific to an isolated IL-17 homolog polypeptide as described herein, the generating done by recombinant, synthetic and/or hybridoma techniques.

The present invention also provides an IL-17 antibody or fragment produced by a method as described herein.

The present invention also provides a method for identifying compounds that bind an IL-17 homolog polypeptide, comprising admixing at least one isolated IL-17 homolog polypeptide as described herein with a test compound or composition; and detecting at least one binding interaction between the polypeptide and the compound or composition.

DESCRIPTION OF THE FIGURES

Figure 1 shows a graph demonstrating an increased number of neutrophils, white blood cells and eosinophils in the blood and other tissues as compared to controls (C) in mice injected with cells retrovirally transduced with DNA encoding IL-17h.

Figure 2 shows a graph demonstrating an increased number of platelets in the blood and other tissues as compared to controls in mice injected with cells retrovirally transduced with DNA encoding IL-17h.

Figure 3 shows increased burst-forming unit-erythroid (BFU-E), colony-forming unit-granulocyte, macrophage (CFU-GM), and colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM) cell types in human bone marrow cells cultured in medium containing mature IL-17h, erythropoietin, IL-3, and stem cell factor as compared to control medium.

Figure 4 shows that mature IL-17h potentiates activity of stem cell factor on colony formation of murine bone marrow granulocyte, macrophage cells.

Figure 5 shows that mature IL-17h polypeptide upregulates ICAM-1 on Normal Human Dermal Fibroblasts as compared to control (medium).

Figure 6 shows that mature IL-17h polypeptide increases IL-6 secretion from Normal Human Dermal Fibroblasts as compared to control (cells only).

Figure 7 shows that mature IL-17h polypeptide increases IL-8 secretion from Normal Human Dermal Fibroblasts as compared to control (cells only).

DESCRIPTION OF THE INVENTION

The present invention provides isolated, recombinant and/or synthetic nucleic acid molecules comprising a polynucleotide encoding IL-17 homolog polypeptides comprising specific fragments and specified variants of such polypeptides, and methods of making and using thereof. An IL-17 homolog polypeptide of the invention comprises at least one fragment domain, and/or specified variant as a portion or fragment of an IL-17 protein as described herein. The present invention also provides methods for making and using such polypeptides for treating various pathologies or abnormal cell conditions in cells, tissues, organs or organisms, potentiation of hematopoietic cell growth, such as in bone marrow, spleen, lymph tissue or other organs or tissues such as blood, as well as enhancing various immune responses or systems or cells, as well as improving or treating inflammatory conditions.

UTILITY

The present invention also provides at least one utility by providing isolated nucleic acid comprising polynucleotides of sufficient length and complementarity to an IL-17 nucleic acid to use as probes or amplification primers in the detection, quantitation, or isolation of gene transcripts. For example, isolated nucleic acids of the present invention can be used as probes in detecting deficiencies in the level of mRNA in screenings for detecting mutations in the gene (e.g., substitutions, deletions, or additions), for monitoring upregulation of expression or changes in biological activity as described herein in screening assays of compounds, and/or for detection of any number of allelic variants (polymorphisms) of the gene.

The isolated nucleic acids of the present invention can also be used for recombinant expression of IL-17 homolog polypeptides, or for use as immunogens in the preparation and/or screening of antibodies. The isolated nucleic acids of the present invention can also be employed for use in sense or antisense suppression of one or more IL-17 genes, nucleic acids or genes in a host cell, or tissue. Attachment of chemical agents which bind, intercalate, cleave and/or crosslink to the isolated nucleic acids of the present invention can also be used to modulate transcription or translation of at least one nucleic acid.

IL-17 homolog polypeptides, optionally in combination with other cytokines, can also be used for accelerating hematopoietic recovery after cancer therapy; modulating, *e.g.*, stimulating, an increase in platelet production via increased differentiation of megakaryocytes from progenitor cells and/or an increase in proliferation of megakaryocytes; treating chemotherapy-induced blood disorders and in bone

marrow transplantation; treating patients receiving immunosuppressive agents after organ transplantation; treating AIDS-related bone marrow failure and opportunistic infections; treating inflammatory diseases of at least one of the gastrointestinal system, joints, airway/lungs and other related or similar diseases.

An IL-17 homolog polypeptide of the present invention further stimulates human dermal fibroblasts and other cells, tissues, organs and mammals, to secrete IL-6 and/or IL-8, as well as enhances ICAM-1 expression in human HS-27 fibroblast and human dermal fibroblast cells, as well as other cells, tissues, organs and mammals. It is understood that the above listing of utilities is not intended to be an exhaustive list. Further utilities are described elsewhere herein and even further utilities would be immediately apparent to the skilled artisan.

DEFINITIONS

A "polynucleotide" comprises at least 5 - 10 nucleotides of a nucleic acid (RNA, DNA or combination thereof), provided by any means, such as synthetic, recombinant isolation or purification method steps.

A "polypeptide" comprises two or more amino acids linked by peptide bonds.

The terms "complementary" or "complementarity" as used herein refer to the capacity of purine, pyrimidine, synthetic or modified nucleotides to associate by partial or complete complementarity through hydrogen or other bonding to form partial or complete double or triple stranded nucleic acid molecules. The following base pairs occur by complete complementarity: (i) guanine (G) and cytosine (C); (ii) adenine (A) and thymine (T); and adenine (A) and uracil (U). "Partial complementarity" refers to association of two or more bases by one or more hydrogen bonds or attraction

that is less than the complete complementarity as described above. Partial or complete complementarity can occur between any two nucleotides, including naturally occurring or modified bases, e.g., as listed in 37 CFR §1.822. All such nucleotides are included in polynucleotides of the invention as described herein.

"Fragment" refers to a fragment, piece, portion, or sub-region of a nucleic acid or polypeptide molecule as disclosed herein, such that the fragment comprises 4 or more amino acids, or 10 or more nucleotides, that are contiguous in the referenced polypeptide or nucleic acid molecule. A fragment thereof may or may not retain biological activity. For example, a fragment of a polypeptide disclosed herein could be used as an antigen to raise a specific antibody against the referenced polypeptide molecule. In a preferred embodiment, fragments of the IL-17 homolog polypeptide have "IL-17 homolog polypeptide activity" as defined below. In a further preferred embodiment, fragments of nucleic acids as described herein encode polypeptides having "IL-17 homolog polypeptide activity."

The term "fusion protein" denotes a hybrid protein molecule not found in nature comprising a translational fusion or enzymatic fusion in which two or more different proteins or fragments thereof are covalently linked on a single polypeptide chain. The term "polypeptide" also includes such fusion proteins.

"Host cell" refers to any eucaryotic, procaryotic, or fusion or other cell or pseudo cell or membrane containing construct that is suitable for propagating and/or expressing an isolated nucleic acid that is introduced into the host cell by any suitable means known in the art (e.g., but not limited to, transformation or transfection, or the like).

The cell can be part of a tissue or organism, isolated in culture or in any other suitable form.

The term "hybridization" as used herein refers to a process in which a partially or completely single-stranded nucleic acid molecule joins with a complementary strand through nucleotide base pairing. Hybridization can occur under conditions of low, moderate to high stringency, with high stringency preferred. The degree of hybridization depends upon, for example, the degree of homology, the stringency conditions, and the length of hybridizing strands.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA, RNA, or both which has been removed from its native or naturally occurring environment. For example, recombinant nucleic acid molecules contained or generated in culture, a vector and/or a host cell are considered isolated for the purposes of the present invention. Further examples of isolated nucleic acid molecules include recombinant nucleic acid molecules maintained in heterologous host cells or purified (partially or substantially) nucleic acid molecules in solution. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the nucleic acid molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically, purified from or provided in cells containing such nucleic acids, where the nucleic acid is other than a naturally occurring form, quantitatively or qualitatively.

"Isolated" used in reference to at least one polypeptide of the invention describes a state of isolation such that the peptide or polypeptide is not in a naturally occurring form and/or has been purified to remove at least some portion of cellular or non-cellular molecules with

which the protein is naturally associated. However, "isolated" may include the addition of other functional or structural polypeptides for a specific purpose, where the other peptide may occur naturally associated with at least one polypeptide of the present invention.

"Isolated" used in reference to an antibody that binds an epitope specific to a polypeptide of the invention describes a state of isolation such that the antibody is not in a naturally occurring form and/or has been purified to remove at least some portion of cellular or non-cellular molecules with which it is naturally associated. However, "isolated" may include the addition of some molecules that are naturally associated with at least one antibody of the present invention.

A "nucleic acid probe," "oligonucleotide probe," or "probe" as used herein comprises at least one detectably labeled or unlabeled nucleic acid which hybridizes under specified hybridization conditions with at least one other nucleic acid. This term also refers to a single or partially double stranded nucleic acid, oligonucleotide or polynucleotide that will associate with a complementary or partially complementary target nucleic acid to form at least a partially double-stranded molecule. A nucleic acid probe may be an oligonucleotide or a nucleotide polymer. A probe can optionally contain a detectable moiety which may be attached to the end(s) of the probe or be internal to the sequence of the probe, termed a "detectable probe" or "detectable nucleic acid probe."

A "primer" is a nucleic acid fragment which functions as an initiating substrate for enzymatic or synthetic elongation of, for example, a nucleic acid molecule, e.g., using a amplification reaction, such as, but not limited to, a polymerase chain reaction (PCR), as known in the art.

The term "promoter" refers to a nucleic acid sequence that directs the initiation of transcription, for example, of DNA to RNA. An inducible promoter is one that is regulatable by environmental signals, such as carbon source, heat, or metal ions, for example.

The term "stringency" refers to hybridization conditions for nucleic acids in solution. High stringency conditions disfavor non-homologous base pairing. Low stringency conditions have much less of this effect. Stringency may be altered, for example, by temperature and salt concentration, or other conditions, as well known in the art.

As used herein, "stringent conditions" refers to preferably (a) a temperature of about 42° C , a formamide concentration of about 20%, and a low salt (5X SSC); or, alternatively, a temperature of about 65° C, or less, and a low salt (5X SSPE) concentration; or (b) hybridization in 0.5 M NaHPO4, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C (See, e.g., Ausubel et al., ed., Current Protocols in Molecular Biology, 1987-1998, Wiley Interscience, New York, at §2.10.3). "SSC" comprises a hybridization and wash solution. A stock 20X SSC solution contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0. "SSPE" comprises a hybridization and wash solution. A 1X SSPE solution contains 180 mM NaCl, 9mM Na2HPO4, 0.9 mM Na2HPO4 and 1 mM EDTA, pH 7.4.

The term "vector" as used herein refers to a nucleic acid compound used for introducing exogenous or endogenous nucleic acid into host cells. A vector comprises a nucleotide sequence which may encode one or more polypeptide molecules. Plasmids, cosmids, viruses, and bacteriophages, in a natural state or which have undergone recombinant engineering, are non-limiting examples of commonly used

vectors to provide recombinant vectors comprising at least one desired isolated nucleic acid molecule.

The various restriction enzymes disclosed and described herein are commercially available and the manner of use of the enzymes including reaction conditions, cofactors, and other requirements for activity are well known to one of ordinary skill in the art (New England Biolabs, Boston; Life Technologies, Rockville, Md.). Reaction conditions for particular enzymes are preferably carried out according to the manufacturer's recommendation.

"IL-17 homolog polypeptide(s)" as used herein refers to the polypeptide shown in SEQ ID NO:2 as well as fragments and variants thereof. "Fragments" are described above. "Variants" are polypeptides where one or more amino acid of SEQ ID NO:2 has been substituted or deleted or where one or more amino acid has been inserted into SEQ ID NO:2. Variants also include polypeptides having at least an 80% sequence identity to SEQ ID NO:2. Variants also include polypeptides where one or more amino acids of SEQ ID NO: 2 is replaced with a conserved amino acid. Variants also include polypeptides where one or more glycosylation sites have been added. Variants also include fusion proteins comprising SEQ ID NO:2 as described herein. Variants also include polypeptides encoded by nucleotides that hybridize to SEQ ID NO:1. Variants further include mature IL-17 homolog polypeptides. "Mature IL-17 homolog polypeptide(s)" refers to a IL-17 homolog polypeptide that lacks a signal (or leader) peptide. For example, a mature IL-17 homolog polypeptide lacks amino acids 19-197 of SEQ ID NO:2. This mature polypeptide lacks the signal peptide shown by amino acids 1-18 of SEQ ID NO:2. Variants further include species homologs or intra-species polymorphisms that can be identified via the techniques described herein.

The term "IL-17 homolog polypeptide activity" as used herein refers to polypeptides having one or more of the activities described herein for the IL-17 homolog polypeptide, such as the ability to stimulate hematopoiesis, or the ability to stimulate an increase in platelet cells. "IL-17 homolog polypeptide activity" also includes an activity of at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95% - 100% of the mature IL-17 homolog polypeptide(amino acids 19-197 of SEQ ID NO:2).

The designation "IL-17h" refers to a polypeptide comprising the amino acid sequence shown in SEQ ID NO:2. The designation "mature IL-17h" refers to a polypeptide comprising amino acids 19-197 of SEQ ID NO:2.

The use of the singular herein, for example, "a" IL-17 homolog polypeptide, "the" IL-17 homolog polypeptide, or "said" IL-17 homolog polypeptide is used for convenience of expression only and is not intended to restrict the described term to the singular. That is, as used herein, the singular is intended to mean "at least one" and does not connote "only one" unless the contrary is explicitly stated.

Use of "or" herein connotes "and/or" unless otherwise indicated.

NUCLEIC ACID MOLECULES

Using the information provided herein, such as the nucleotide sequences encoding at least a 3-50 amino acid fragment of SEQ ID NO:2, a nucleic acid molecule of the present invention encoding an IL-17 homolog polypeptide can be obtained using well-known cloning and/or screening procedures, such as those for cloning cDNAs using mRNA as starting material. The identified nucleotide sequence of an IL-17 nucleic acid of SEQ ID NO:1 contains an open reading frame encoding a polypeptide of 197 amino acid residues (SEQ ID NO:2). The first 18 amino acids of SEQ ID NO:2 have been identified as a signal peptide.

As indicated, nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combination thereof. The DNA can be triple-, double-stranded or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

Isolated nucleic acid molecules of the present invention include nucleic acid molecules comprising an open reading frame (ORF) shown in SEQ ID NO:1; nucleotides 55-591 of SEQ ID NO:1 which encode the mature polypeptide; and nucleic acid molecules which comprise a nucleotide sequence substantially different from SEQ ID NO:1, but which, due to the degeneracy of the genetic code, still encode at least one IL-17 homolog polypeptide as described and enabled herein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate nucleic acid variants that code

for specific IL-17 homolog polypeptides of the present invention (See, e.g., Ausubel et al.).

In a further embodiment, nucleic acid molecules are provided encoding the full-length IL-17 homolog polypeptide lacking the N-terminal methionine. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly nucleic acid molecules, are useful as probes for gene mapping, by in situ hybridization with chromosomes, and for detecting transcription, translation and/or expression of the IL-17 gene in human tissue, for instance, by Northern blot analysis for mRNA detection.

Moreover, both the DNA and protein molecules of the invention can be defined with reference to "sequence identity." As used herein, "sequence identity" refers to a comparison made between two molecules using standard algorithms well known in the art. The preferred algorithm for calculating sequence identity for the present invention is the Smith-Waterman algorithm, where SEQ ID NO:1 is used as the reference sequence to define the percentage identity of polynucleotide homologs over its length. The choice of parameter values for matches, mismatches, and inserts or deletions is arbitrary, although some parameter values have been found to yield more biologically realistic results than others. One preferred set of parameter values for the Smith-Waterman algorithm is set forth in the "maximum similarity segments" approach, which uses values of 1 for a matched residue and -1/3 for a mismatched residue (a residue being a either a single nucleotide or single amino acid) (Waterman, Bulletin of Mathematical Biology 46:473-500

(1984)). Insertions and deletions (indels), x, are weighted as

$$x_k = 1 + k/3,$$

where k is the number of residues in a given insert or deletion (Id.).

For instance, a sequence that was identical to the 197 amino acid residue sequence of SEQ ID NO:2, except for 18 amino acid substitutions and an insertion of 3 amino acids, would have a percent identity given by:

[(1 x 179 matches) - (⅓ x 18 mismatches)
- (1 + 3/3 indels)] / 197 = 87% identity

Preferred nucleic acid molecules are those having at least about 80%-100% sequence identity to SEQ ID NO:1. Particularly preferred molecules have at least about 90%-100% sequence identity to SEQ ID NO:1. Even more preferred molecules have at least about 95%-100% sequence identity, and most preferred molecules have at least 98%-100% sequence identity to SEQ ID NO:1.

The nucleic acid molecules of the present invention further include molecules having substitutions, insertions, or deletions of one or more of the nucleotides of SEQ ID NO:1. Such nucleotide substitutions, insertions, or deletions include, for example, those which result in codon optimization for a desired host cell or expression system or which result in a substitution of one or more conserved amino acids in the encoded protein.

Nucleic acid molecules of the present invention further include those encoding the amino acid sequence of the mature polypeptide, by itself; the coding sequence for the mature polypeptide and additional sequences, such as the coding sequence of the mature polypeptide, with or without the

aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5′ and 3′ sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example - ribosome binding and stability of mRNA); an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, the sequence encoding a polypeptide can be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide.

NUCLEIC ACID FRAGMENTS

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. A fragment of an isolated nucleic acid molecule is, for example, a nucleic acid molecule having at least 10 nucleotides (nt) of a nucleotide sequence shown in SEQ ID NO:1, and includes fragments of at least about 10 nt, at least about 15 nt, at least about 30 nt, and at least about 40 nt in length, which are useful, inter alia, as diagnostic probes and primers as described herein. Of course, larger fragments such as those of at least about 50, 100, 120, 200, 500-591 nt in length, are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence—as shown in SEQ ID NO:1. By a fragment at least 10 nt in length, for example, is intended fragments which include 10 or more contiguous bases from the nucleotide sequence as shown in SEQ ID NO:1, or consensus sequences thereof, as determined by methods known in the art.

Such nucleotide fragments are useful, for example, according to the present invention for screening DNA sequences that code for one or more fragments of an IL-17 homolog polypeptide as described herein.

Preferred nucleic acid fragments of the present invention also include nucleic acid molecules encoding epitope-bearing portions of an IL-17 homolog polypeptide.

OLIGONUCLEOTIDE AND POLYNUCLEOTIDE PROBES

In another aspect, the invention provides a polynucleotide (either DNA or RNA) that comprises at least about 10 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably at least about 30-200 nt of a nucleic acid molecule described herein. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 10 nt in length," for example, is intended 10 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the nucleotide sequence as shown in SEQ ID NO:1).

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) of an IL-17 homolog polypeptide cDNA shown in SEQ ID NO:1), or to a complementary stretch of T (or U) residues, would not be included in a probe of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The present invention also provides subsequences of full-length nucleic acids which are useful as probes. Any number of subsequences can be obtained by reference to SEQ

ID NO:1, and using primers which selectively amplify, under stringent conditions to: at least two sites to the polynucleotides of the present invention, or to two sites within the nucleic acid which flank and comprise a polynucleotide of the present invention, or to a site within a polynucleotide of the present invention and a site within the nucleic acid which comprises it. A variety of methods for obtaining 5' and/or 3' ends is well known in the art. See, e.g., RACE (Rapid Amplification of Complementary Ends) as described in Frohman, M. A., in PCR Protocols: A Guide to Methods and Applications, M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White, Eds. (Academic Press, Inc., San Diego, 1990), pp. 28-38.); see also, U.S. Pat. No. 5,470,722, and Current Protocols in Molecular Biology, Unit 15.6, Ausubel et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995).

Primer sequences can be obtained by reference to a contiguous subsequence of a polynucleotide of the present invention. Primers are chosen to selectively hybridize, under PCR amplification conditions, to a polynucleotide of the present invention in an amplification mixture comprising a genomic and/or cDNA library from the same species. Generally, the primers are complementary to a subsequence of the amplicon they yield. In some embodiments, the primers will be constructed to anneal at their 5' terminal ends to the codon encoding the carboxy or amino terminal amino acid residue (or the complements thereof) of the polynucleotides of the present invention. The primer length in nucleotides is selected from the group of integers consisting of from at least 15 to 50. Thus, the primers can be at least 15, 18, 20, 25, 30, 40, or 50 nucleotides in length. A non-annealing sequence at the 5'end of the primer (a "tail") can

be added, for example, to introduce a cloning site at the terminal ends of the amplicon.

The amplification primers may optionally be elongated in the 3′ direction with additional contiguous nucleotides from the polynucleotide sequences, such as SEQ ID NO: 1, from which they are derived. The number of nucleotides by which the primers can be elongated is selected from the group of integers consisting of from at least 1 to at least 25. Thus, for example, the primers can be elongated with an additional 1, 5, 10, or 15 nucleotides. Those of skill will recognize that a lengthened primer sequence can be employed to increase specificity of binding (i.e., annealing) to a target sequence.

The amplification products can be translated using expression systems well known to those of skill in the art and as discussed, infra. The resulting translation products can be confirmed as polypeptides of the present invention by, for example, assaying for the appropriate catalytic activity (e.g., specific activity and/or substrate specificity), or verifying the presence of one or more linear epitopes which are specific to a polypeptide of the present invention. Methods for protein synthesis from PCR derived templates are known in the art and available commercially (See, e.g., Amersham Life Sciences, Inc., Catalog '97, p.354).

POLYNUCLEOTIDES WHICH SELECTIVELY HYBRIDIZE TO A POLYNUCLEOTIDE AS DESCRIBED HEREIN

As indicated above, the present invention provides isolated IL-17 homolog polynucleotides that hybridize to a nucleic acid of the present invention. For example, the present invention provides nucleic acids that hybridize to, for example, SEQ ID NO: 1. Preferably the hybridization

conditions are stringent as defined above. Thus, SEQ ID NO: 1 or other polynucleotides of the invention can be used for isolating, detecting, and/or quantifying nucleic acids that hybridize thereto. For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated from, or otherwise complementary to, a cDNA from a nucleic acid library.

Preferably, the cDNA library comprises at least 80% full-length sequences, preferably at least 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

Optionally, the polynucleotides of this embodiment will share an epitope with a polypeptide encoded by the polynucleotides described above. Thus, these polynucleotides encode a first polypeptide which elicits production of antisera comprising antibodies which are specifically reactive to a second polypeptide encoded by a polynucleotide described above. The polynucleotides of this embodiment embrace nucleic acid sequences which can be employed for selective hybridization to a polynucleotide encoding a polypeptide of the present invention.

Screening polypeptides for specific binding to antisera can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure. Antibody screening of peptide display libraries is well known in the art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 15 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT patent publication Nos. 91/17271, 91/18980, 91/19818, and 93/08278. Other systems for generating libraries of peptides have aspects of both in vitro chemical synthesis and recombinant methods. See, PCT Patent publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Patent Nos. 5,658,754; and 5,643,768. Peptide display libraries, vector, and screening kits are commercially available from such suppliers as Invitrogen (Carlsbad, CA).

COMPLEMENTARY POLYNUCLEOTIDES

As indicated above, the present invention provides isolated nucleic acids comprising IL-17 homolog polynucleotides, wherein the polynucleotides are complementary to the polynucleotides described herein, above. As those of skill in the art will recognize, complementary sequences base-pair throughout the entirety of their length with such polynucleotides (i.e., have 100%

sequence identity over their entire length). Complementary bases associate through hydrogen bonding in double stranded nucleic acids. For example, the following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and uracil.

CONSTRUCTION OF NUCLEIC ACIDS

The isolated nucleic acids of the present invention can be made using (a) standard recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well known in the art.

The nucleic acids may conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites may be inserted into the nucleic acid to aid in isolation and manipulation of the polynucleotide. Also, translatable sequences may be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. The nucleic acid of the present invention - excluding the polynucleotide sequence - is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention.

Additional sequences may be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Typically, the length of a nucleic acid of the present invention less the length of its polynucleotide of the present invention is less than 20 kilobase pairs, often less than 15 kb, and frequently less

than 10 kb. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art.

RECOMBINANT METHODS FOR CONSTRUCTING NUCLEIC ACIDS

The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or a hybrid thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes which selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. Isolation of RNA, and construction of cDNA and genomic libraries is well known to those of ordinary skill in the art.

NUCLEIC ACID SCREENING AND ISOLATION METHODS

The cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the present invention such as those disclosed herein. Probes may be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide.

For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100 percent; however, it should be understood that minor sequence variations in the probes and primers may be compensated for by reducing the stringency of the hybridization and/or wash medium.

Methods of amplification of RNA or DNA are well known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein. According to the present invention, the use of nucleic acids encoding portions of an IL-17 homolog polypeptide according to the present invention, as amplification primers, allows for advantages over known amplification primers, due to the increase in sensitivity, selectivity and/or rate of amplification.

Known methods of DNA or RNA amplification include, but are not limited to polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. patent Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis et al.; 4,795,699 and 4,921,794 to Tabor et al.; 5,142,033 to Innis; 5,122,464 to Wilson et al.; 5,091,310 to Innis; 5,066,584 to Gyllensten et al.; 4,889,818 to Gelfand et al.; 4,994,370 to Silver et al.; 4,766,067 to Biswas; 4,656,134 to Ringold) and RNA mediated amplification which uses anti-sense RNA to the target sequence as a template for double stranded DNA synthesis (U.S. patent No. 5,130,238 to Malek et al., with the tradename NASBA).

For instance, PCR technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through in vitro amplification methods are found in Berger, Sambrook, and Ausubel, as well as Mullis et al., U.S. Patent No. 4,683,202 (1987); and, PCR Protocols A Guide to Methods and Applications, Innis et al., Eds., Academic Press Inc., San Diego, CA (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). The T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

SYNTHETIC METHODS FOR CONSTRUCTING NUCLEIC ACIDS

The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by methods such as the phosphotriester method of Narang et al., Meth. Enzymol. 68: 90 (1979); the phosphodiester method of Brown et al., Meth. Enzymol. 68:109 (1979); the diethylphosphoramidite method of Beaucage et al., Tetra. Lett. 22:1859 (1981); the solid phase phosphoramidite triester method described by Beaucage et al., Tetra. Letts. 22(20):1859 (1981), e.g., using an automated synthesizer, e.g., as described in Needham-VanDevanter et al., Nucleic Acids Res., 12:6159 (1984); and, the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis generally produces a single stranded oligonucleotide. This may be

converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill will recognize that while chemical synthesis of DNA is limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

RECOMBINANT EXPRESSION CASSETTES

The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence coding for the desired polynucleotide of the present invention, for example a cDNA or a genomic sequence encoding a full-length polypeptide of the present invention, can be used to construct a recombinant expression cassette which can be introduced into the desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences which will direct the transcription of the polynucleotide in the intended host cell.

Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention. These promoters can also be used, for example, in recombinant expression cassettes to drive expression of antisense nucleic acids to reduce, increase, or alter IL-17 content and/or composition in a desired tissue.

In some embodiments, isolated nucleic acids which serve as promoter or enhancer elements can be introduced in the appropriate position (generally upstream) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a

polynucleotide of the present invention. For example, endogenous promoters can be altered in vivo by mutation, deletion, and/or substitution.

A polynucleotide of the present invention can be expressed in either the sense or anti-sense orientation as desired. Expression in the anti-sense orientation is a recognized means for blocking transcription of a target gene. Moreover, introduction of multiple copies of nucleic acid configured in the sense orientation can be an effective way to block transcription.

A variety of cross-linking agents, alkylating agents and radical generating species as pendant groups on polynucleotides of the present invention can be used to bind, label, detect, and/or cleave nucleic acids. For example, Vlassov et al., Nucleic Acids Res. 14:4065 (1986), describe covalent bonding of a single-stranded DNA fragment with alkylating derivatives of nucleotides complementary to target sequences. A report of similar work by the same group is that by Knorre et al., Biochimie 67:785 (1985). Iverson et al., J. Am. Chem. Soc. 109:1241 (1987), also showed sequence-specific cleavage of single-stranded DNA mediated by incorporation of a modified nucleotide which was capable of activating cleavage. Meyer et al., J. Am. Chem. Soc. 111:8517 (1989), effect covalent crosslinking to a target nucleotide using an alkylating agent complementary to the single-stranded target nucleotide sequence. A photoactivated crosslinking to single-stranded oligonucleotides mediated by psoralen was disclosed by Lee et al., Biochemistry 27:3197 (1988). Use of crosslinking in triple-helix forming probes was also disclosed by Home et al., J. Am. Chem. Soc. 112:2435 (1990). Use of N4, N4-ethanocytosine as an alkylating agent to crosslink to single-stranded oligonucleotides has also been described by Webb et al., J.

Am. Chem. Soc. 108:2764 (1986); Nucleic Acids Res. 14:7661 (1986); Feteritz et al., J. Am. Chem. Soc. 113:4000 (1991). Various compounds to bind, detect, label, and/or cleave nucleic acids are known in the art. See, for example, U.S. Patent Nos. 5,543,507; 5,672,593; 5,484,908; 5,256,648; and, 5,681941.

VECTORS AND HOST CELLS

The present invention also relates to vectors which include isolated nucleic acid molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and methods for the production of IL-17 homolog polypeptides or fragments thereof by recombinant techniques, as well known in the art. See, e.g., Sambrook et al., 1989; Ausubel, et al., 1987-1989, each entirely incorporated herein by reference.

The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation

initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include, e.g., dihydrofolate reductase or neomycin resistance for eucaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art. Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eucaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 1-4 and 16-18; Ausubel, supra, Chapters 1, 9, 13, 15, 16.

The polypeptide can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of a polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to a polypeptide to facilitate purification. Such regions can be removed prior to final preparation of a polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.29-17.42 and 18.1-18.74; Ausubel, supra, Chapters 16, 17 and 18.

EXPRESSION OF PROTEINS IN HOST CELLS

Using nucleic acids of the present invention, one may express a protein of the present invention in a recombinantly engineered cell such as bacterial, yeast, insect, or mammalian cells. The cells produce the protein in a non-natural condition (e.g., in quantity, composition, location, and/or time), because they have been genetically altered through human intervention to do so.

It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes will be made.

In brief summary, the expression of isolated nucleic acids encoding a protein of the present invention will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding a protein of the present invention. To obtain high level expression of a cloned gene, it is desirable to construct expression vectors which contain, at the minimum, a strong promoter to direct transcription, a ribosome binding site for translational initiation, and a transcription/translation terminator. One of skill would recognize that modifications can be made to a protein of the present invention without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, or additional amino acids (e.g., poly His) placed on either terminus to create conveniently located restriction sites or termination codons or purification sequences.

EXPRESSION IN PROKARYOTES

Prokaryotic cells may be used as hosts for expression. Prokaryotes most frequently are represented by various strains of E. coli; however, other microbial strains may also be used. Commonly used prokaryotic control sequences which are defined herein to include promoters for

transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta lactamase (penicillinase) and lactose (lac) promoter systems (Chang et al., Nature 198:1056 (1977)), the tryptophan (trp) promoter system (Goeddel et al., Nucleic Acids Res. 8:4057 (1980)) and the lambda derived PL promoter and N-gene ribosome binding site (Shimatake et al., Nature 292:128 (1981)). The inclusion of selection markers in DNA vectors transfected in E. coli is also useful. Examples of such markers include genes specifying resistance to ampicillin, tetracycline, or chloramphenicol.

The vector is selected to allow introduction into the appropriate host cell. Bacterial vectors are typically of plasmid or phage origin. Appropriate bacterial cells are infected with phage vector particles or transfected with naked phage vector DNA. If a plasmid vector is used, the bacterial cells are transfected with the plasmid vector DNA. Expression systems for expressing a protein of the present invention are available using Bacillus sp. and Salmonella. (Palva et al., Gene 22:229 (1983); Mosbach et al., Nature 302:543 (1983).

EXPRESSION IN EUKARYOTES

A variety of eukaryotic expression systems such as yeast, insect cell lines, plant and mammalian cells, are known to those of skill in the art. As explained briefly below, an IL-17 homolog polypeptide of the present invention can be expressed in these eukaryotic systems.

Synthesis of heterologous proteins in yeast is well known. Sherman, F., et al., Methods in Yeast Genetics, Cold Spring Harbor Laboratory (1982) is a well recognized work describing the various methods available to produce the

protein in yeast. Two widely utilized yeast for production of eukaryotic proteins are Saccharomyces cerevisiae and Pichia pastoris. Vectors, strains, and protocols for expression in Saccharomyces and Pichia are known in the art and available from commercial suppliers (e.g., Invitrogen). Suitable vectors usually have expression control sequences, such as promoters, including 3-phosphoglycerate kinase or alcohol oxidase, and an origin of replication, termination sequences and the like as desired.

A protein of the present invention, once expressed, can be isolated from yeast by lysing the cells and applying standard protein isolation techniques to the lysates. The monitoring of the purification process can be accomplished by using Western blot techniques or radioimmunoassay of other standard immunoassay techniques.

The sequences encoding proteins of the present invention can also be ligated to various expression vectors for use in transfecting cell cultures of, for instance, mammalian, insect, or plant origin. Illustrative of cell cultures useful for the production of the peptides are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions may also be used. A number of suitable host cell lines capable of expressing intact proteins have been developed in the art, and include the HEK293, AV12, BHK21, and CHO cell lines. HEK293 and AV12 are preferred cell lines. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter (e.g., the CMV promoter, a HSV tk promoter or pgk (phosphoglycerate kinase) promoter), an enhancer (Queen et al., Immunol. Rev. 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites

(e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. Other animal cells useful for production of proteins of the present invention are available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (7th edition, 1992).

Appropriate vectors for expressing proteins of the present invention in insect cells are usually derived from the SF9 baculovirus. Suitable insect cell lines include mosquito larvae, silkworm, armyworm, moth and Drosophila cell lines such as a Schneider cell line (See Schneider, J. Embryol. Exp. Morphol. 27:353 (1987).

As with yeast, when higher animal or plant host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript may also be included. An example of a splicing sequence is the VP1 intron from SV40. (Sprague et al., J. Virol. 45:773 (1983). Additionally, gene sequences to control replication in the host cell may be incorporated into the vector such as those found in bovine papilloma virus type-vectors. Saveria-Campo, M., Bovine Papilloma Virus DNA a Eukaryotic Cloning Vector in DNA Cloning Vol. II a Practical Approach, D.M. Glover, Ed., IRL Press, Arlington, Virginia pp. 213-238 (1985).

EXPRESSED PROTEIN PURIFICATION

An IL-17 homolog polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography,

phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eucaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention can be glycosylated or can be non-glycosylated. In addition, polypeptides of the invention can also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.37-17.42; Ausubel, supra, Chapters 10, 12, 13, 16, 18 and 20.

IL-17 HOMOLOG POLYPEPTIDES AND FRAGMENTS AND VARIANTS

The invention further provides an isolated IL-17 homolog polypeptides having fragments or specified variants of the amino acid sequence encoded by the amino acid sequence in SEQ ID NO:2.

The isolated proteins of the present invention comprise a polypeptide having at least 5 - 10 amino acids encoded by any one of the polynucleotides of the present invention as discussed more fully, supra, or polypeptides which are conservatively modified variants thereof.

An exemplary polypeptide sequence is provided in the 197 amino acid sequence shown in SEQ ID NO:2. The proteins of the present invention or variants thereof can comprise any number of contiguous amino acid residues from a

polypeptide of the present invention, wherein that number is selected from the group of integers consisting of from 10 to the number of residues in a full-length IL-17 homolog polypeptide. Optionally, this sequence of contiguous amino acids is at least 15, 20, 25, 30, 35, or 40 amino acids in length, often at least 50, 60, 70, 80, or 90 amino acids in length. Further, the number of such sequences can be any integer selected from the group consisting of from 1 to 20, such as 2, 3, 4, or 5.

The IL-17 homolog polypeptides further include polypeptides having at least about 80%-100% sequence identity to SEQ ID NO:2 or to amino acids 19-197 of SEQ ID NO:2. Particularly preferred molecules have at least about 90%-100% sequence identity to SEQ ID NO:2 or to amino acids 19-197 of SEQ ID NO:2. Even more preferred molecules have at least about 95%-100% sequence identity, and most preferred molecules have at least 98%-100% sequence identity to SEQ ID NO:2 or to amino acids 19-197 of SEQ ID NO:2. The parameters for sequence identity are provided above.

Generally, the polypeptides of the present invention will, when presented as an immunogen, elicit production of an antibody specifically reactive to a polypeptide of the present invention encoded by a polynucleotide of the present invention as described, supra. Exemplary polypeptides include those which are full-length, such as SEQ ID NO:2. Further, the proteins of the present invention will not bind to antisera raised against a polypeptide of the present invention which has been fully immunosorbed with the same polypeptide. Immunoassays for determining binding are well known to those of skill in the art. A preferred immunoassay is a competitive immunoassay as discussed, infra. Thus, the proteins of the present invention can be employed as immunogens for constructing antibodies immunoreactive to a

protein of the present invention for such exemplary utilities as immunoassays or protein purification techniques.

An IL-17 homolog polypeptide of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein.

Deletional variants include polypeptides where 1, 2, or 3 amino acids have been deleted from either the N-terminus or C-terminus of the mature form of SEQ ID NO:2. The number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions for any given IL-17 homolog polypeptide will not be more than 20, 10, 5, or 3, such as 1-20 or any range or value therein, as specified herein. In one aspect of the invention, the substitutions include one or more conservative substitutions. A "conservative" substitution denotes the replacement of an amino acid residue by another, biologically active similar residue. Examples of conservative substitution include the substitution of one hydrophobic residue, such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like.

Further exemplary modifications of IL-17 homolog polypeptides of the present invention can be made in accordance with the following Table. Such modifications are expected to retain the biological activity of the parent molecule based on the art recognized substitutability of certain amino acids (See e.g. M. Dayhoff, In Atlas of Protein Sequence and Structure, Vol. 5, Supp. 3, pgs 345-352, 1978).

ORIGINAL RESIDUE	EXEMPLARY SUBSTITUTIONS
ALA	SER, THR
ARG	LYS
ASN	HIS, SER
ASP	GLU, ASN
CYS	SER
GLN	ASN, HIS
GLU	ASP, GLU
GLY	ALA, SER
HIS	ASN, GLN
ILE	LEU, VAL, THR
LEU	ILE, VAL
LYS	ARG, GLN, GLU, THR
MET	LEU, ILE, VAL
PHE	LEU, TYR
SER	THR, ALA, ASN
THR	SER, ALA
TRP	ARG, SER
TYR	PHE
VAL	ILE, LEU, ALA
PRO	ALA

Amino acids in an IL-17 homolog polypeptide of the present invention that are essential for function can be identified by methods known in the art, such as site-

directed mutagenesis or alanine-scanning mutagenesis. (Cunningham et al., Science 244:1081 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as protein binding or apoptosis and/or transforming activity or activity related to enhancing(e.g., stimulating) hematopoiesis, expression of IL-6, IL-8, or enhancing (e.g., stimulating) surface presentation of ICAM-1. Sites that are critical for ligand-protein binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith et al., J. Mol. Biol. 224:899 (1992) and de Vos et al., Science 255:306 (1992)).

An IL-17 homolog polypeptide can further comprise from 1 to 197 contiguous amino acids of SEQ ID NO:2.

An IL-17 homolog polypeptide further includes an amino acid sequence comprising SEQ ID NO:2 or amino acids 19-197 of SEQ ID NO:2.

The present invention further provides IL-17 homolog polypeptides that enhance, e.g., stimulate, expression and/or secretion of IL-6, IL-8, and ICAM-1 in animal cells. Such activity can be assayed, for example, by IL-17 homolog polypeptide assays that include, for example, at least one of the following, as described herein or as known in the art:

IL-6 secretion by human dermal fibroblasts

R&D Systems Bioassay for IL-17 activity using human dermal fibroblasts.

IL-6 secretion by HS-27 human foreskin fibroblast cells

HS-27 human foreskin fibroblasts (ATTC HTB-38) were trypsinized from a 90% confluent flask, washed once, and

resuspended in DMEM + 10%FBS at $1x10^5$ cells/ml. 10^4 (100µl) cells were added per well in a 96 well tissue culture treated plate along with 100µl /well of IL-17 or mature IL-17h protein. The cells were incubated for 48 hours, after which 100µl cell free supernatant was assayed for IL-6 using the R&D Systems human IL-6 Quantikine.

IL-8 secretion by human dermal fibroblasts

R&D Systems Bioassay for IL-17 using human dermal fibroblasts with substitution of the human IL-8 Quantikine for the human IL-6 Quantikine

ICAM-1 expression by human dermal fibroblasts

Human dermal fibroblasts from Clonetics were plated at $2x10^5$ cells /well in a 6-well dish in 2ml Fibroblast Basal Medium (Clonetics) + 2% heat inactivated FBS in the presence or absence of IL-17 or mature IL-17h. Cells were incubated for 48 hours, trypsinized in low trypsin (Clonetics), spun down, and resuspended in 50µl 0.1% BSA/PBS. The cells were then stained for 30 minutes at 4°C with either monoclonal anti-human ICAM-1-Fluorescein (R&D Systems BBA20) or anti-mouse IgG1-Fluorescien (isotype control). After washing with 0.1%BSA/PBS, the cells were analyzed by flow cytometric analysis.

ICAM-1 expression by HS-27 human foreskin fibroblast cells

HS-27 human foreskin fibroblasts (ATTC HTB-38) were plated at $2x10^5$ cells /well in a 6-well dish with IL-17 or mature IL-17h in 2ml DMEM + 10%FBS. Cells were incubated for 48 hours, trypsinized in low trypsin (Clonetics), spun down, and resuspended in 50µl 0.1% BSA/PBS. The cells were then stained for 30 minutes at 4°C with either monoclonal anti-human ICAM-1-Fluorescein (R&D Systems BBA20) or anti-

mouse IgG1-Fluorescien (isotype control). After washing with 0.1%BSA/PBS, the cells were analyzed by flow cytometric analysis.

The IL-17 homolog polypeptides of the present invention also include polypeptides corresponding to SEQ ID NO: 2 where 1 or more glycosylation sites are added in order to, for example, increase the half-life of the polypeptide or decrease immunogenicity. Addition of glycosylation sites may be accomplished by altering the amino acid sequence of SEQ ID NO:2. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to SEQ ID NO:2 (for O-linked glycosylation sites). The IL-17 homolog polypeptide amino acid sequences may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the IL-17 homolog polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the IL-17 homolog polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO87/05530 published 11 September 1987, and in Applin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

Another type of covalent modification of IL-17 homolog polypeptides comprises linking the IL-17 homolog polypeptide to one of a variety of non proteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689, 4,301,144; 4,670,417; 4,791,192, or 4,179,337.

The IL-17 homolog polypeptides of the present invention may also be modified in a way to form a chimeric

molecule comprising, for example, fused to another, heterologous polypeptide or amino acid sequence. In one embodiment, such a chimeric molecule comprises a fusion of a IL-17 homolog polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the IL-17 homolog polypeptide. The presence of such epitope-tagged forms of the IL-17 homolog polypeptide can be detected by using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the IL-17 homolog polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag.

IL-17 HOMOLOG POLYPEPTIDE COMPOSITIONS

The present invention also provides IL-17 homolog polypeptide compositions comprising at least one isolated IL-17 homolog polypeptide as described herein.

IL-17 homolog polypeptide compositions of the present invention can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but not limited to, Gennaro, Ed., *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co. (Easton, PA) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the IL-17 homolog composition as well known in the art or as described herein.

Pharmaceutical excipients and additives useful in the present composition include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which may be present singly or in combination. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/polypeptide components, which may also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.

Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose. Preferred powder compositions in accordance with the invention are those that are stable in the absence of sucrose, and particularly those that are stable in the absence of combinations of sucrose and glycine.

IL-17 homolog compositions may also include a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative

buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts such as citrate.

Additionally, the IL-17 homolog polypeptide compositions of the invention may include polymeric excipients/additives such as polyvinylpyrrolidones, Ficolls (a polymeric sugar), dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl-β-cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as "TWEEN 20" and "TWEEN 80"), lipids (*e.g.*, phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA). Other pharmaceutical excipients and/or additives suitable for use in the IL-17 homolog compositions according to the invention are listed in "Remington: The Science & Practice of Pharmacy", 19th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, NJ (1998), the disclosures of which are herein incorporated by reference. Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents.

Therapeutic Applications

An IL-17 homolog composition of the invention is useful, when administered in a therapeutically effective amount, for example, for enhancing hematopoiesis, erythropoiesis, leukopoiesis, thrombocytopoiesis, or for enhancing, e.g., stimulating, production of neutrophils, granulocytes, or platelets by stimulating the proliferation

and/or differentiation of progenitors of such cells, as needed in many different conditions, such as but not limited to, prior to, subsequent to, or during chemotherapy or radiation therapy, in conditions of viral or bacterial infection or in immune related diseases, such as immunodeficient or autoimmune diseases, as known in the art. An IL-17 homolog composition of the invention is also useful, when administered in a therapeutically effective amount, for example, for treatment of conditions associated with (a) inadequate platelet production, such as aplastic anemia, refractory anemias, leukemia, preleukemia/ myelodysplastic syndromes, megaloblastic anemia, chemotherapy or radiation therapy, and organ/bone marrow transplantation (b) increased destruction of platelets, such as idiopathic thrombocytopenia purpura, other immune thrombocytopenias, I-IlV-associated thrombocytopenia, sepsis/disseminated intravascular coagulation, and vasculitis and (c) abnormal platelet function, such as Glanzmann's thrombasthenia, acute/chronic leukemia, myeloproliferative disorders, uremia, platelet storage pool disease, Von Willebrand disease, and postoperative dysfunction(cardiovascular).

Typically, treatment of the above-described conditions is effected by administering therapeutically effective dosages of IL-17 homolog that total, on average, a range from at least about 0.1 to 10000 micrograms IL-17 homolog/kilogram of patient daily, and preferably from at least about .01 to 1 milligram IL-17 homolog/kilogram of patient daily, depending upon the specific activity of IL-17 homolog contained in the composition. Suitable dosages are known to medical practitioners and will, of course, depend upon the particular disease state, specific activity of the composition being administered, and the particular patient

undergoing treatment. In some instances, to achieve the desired therapeutic amount, it may be necessary to provide for repeated administration, *i.e.*, repeated individual inhalations of a particular metered dose, where the individual administrations are repeated until the desired daily dose is achieved.

Modes of Administration

Many known and developing modes of administration can be used according to the present invention for administering pharmaceutically effective amounts of at least one IL-17 homolog composition according to the present invention. While pulmonary administration is used in the following examples, other modes of administration can be used according to the present invention with suitable results.

Non-limiting examples of such administration can include alternative pulmonary, as well as other modes of administration.

IL-17 homolog can be delivered in a carrier, as a solution, emulsion, colloid, or suspension, or as a dry powder, using any of a variety of devices and methods suitable for administration by inhalation described here within or known in the art. Preferably, IL-17 homolog is delivered in a particle size effective for reaching the lower airways of the lung. According to the invention, IL-17 homolog can be delivered by any of a variety of inhalation devices known in the art for administration of a therapeutic agent by inhalation. These devices capable of depositing aerosolized IL-17 homolog formulations in the alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like. Other devices suitable for directing the pulmonary administration of IL-17 homolog are also known in the art.

All such devices require the use of formulations suitable for the administration for the dispensing of IL-17 homolog in an aerosol. Such aerosols can be comprised of either solutions (both aqueous and non aqueous) or solid particles. Metered dose inhalers like the Ventolin® metered dose inhaler, typically use a propellent gas and require actuation during inspiration (WO 94/16970, WO 98/35888 Glaxo). Dry powder inhalers like Turbuhaler™ (Astra), Rotahaler® (Glaxo), Diskus® (Glaxo), Spiros™ inhaler (Dura), devices marketed by Inhale Therapeutics, and the Spinhaler® powder inhaler (Fisons), use breath-actuation of a mixed powder (US 4668218 Astra, EP 237507 Astra, WO 97/25086 Glaxo, WO 94/08552 Dura, US 5458135 Inhale, WO 94/06498 Fisons). Nebulizers like AERx™ Aradigm, the Ultravent® nebulizer (Mallinckrodt), and the Acorn II® nebulizer (Marquest Medical Products) (US 5404871 Aradigm, WO 97/22376), produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc. generate small particle aerosols. These specific examples of commercially available inhalation devices are intended to be representative of specific devices suitable for the practice of this invention, and are not intended as limiting the scope of the invention. There are several desirable features of an inhalation device for administering IL-17 homolog. For example, delivery by the inhalation device is advantageously reliable, reproducible, and accurate. The inhalation device should deliver small particles, e.g. less than about 10 μm, preferably about 1-5 μm, for good respirability.

Administration of IL-17 homolog compositions as a Spray

A spray including IL-17 homolog composition protein can be produced by forcing a suspension or solution of IL-17

homolog composition protein through a nozzle under pressure. The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired output and particle size. An electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of IL-17 homolog composition protein delivered by a sprayer have a particle size less than about 10 μm, preferably in the range of about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm.

Formulations of IL-17 homolog composition protein suitable for use with a sprayer typically include IL-17 homolog composition protein in an aqueous solution at a concentration of about 1 mg to about 20 mg of IL-17 homolog composition protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the IL-17 homolog composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating IL-17 homolog composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating IL-17 homolog composition proteins include sucrose, mannitol, lactose, trehalose, glucose, or the like. The IL-17 homolog composition protein formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the IL-17 homolog composition protein caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the

formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as IL-17 homolog protein can also be included in the formulation.

Administration of IL-17 homolog compositions by a Nebulizer

IL-17 homolog composition protein can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of IL-17 homolog composition protein through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, high-frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of IL-17 homolog composition protein either directly or through a coupling fluid, creating an aerosol including the IL-17 homolog composition protein. Advantageously, particles of IL-17 homolog composition protein delivered by a nebulizer have a particle size less than about 10 μm, preferably in the range of about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm.

Formulations of IL-17 homolog composition protein suitable for use with a nebulizer, either jet or ultrasonic,

typically include IL-17 homolog composition protein in an aqueous solution at a concentration of about 1 mg to about 20 mg of IL-17 homolog composition protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the IL-17 homolog composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating IL-17 homolog composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating IL-17 homolog composition proteins include sucrose, mannitol, lactose, trehalose, glucose, or the like. The IL-17 homolog composition protein formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the IL-17 homolog composition protein caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbital fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as IL-17 homolog protein can also be included in the formulation.

Administration of IL-17 homolog compositions by a Metered Dose Inhaler

In a metered dose inhaler (MDI), a propellant, IL-17 homolog composition protein, and any excipients or other

additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an aerosol, preferably containing particles in the size range of less than about 10 μm, preferably about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm. The desired aerosol particle size can be obtained by employing a formulation of IL-17 homolog composition protein produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant.

Formulations of IL-17 homolog composition protein for use with a metered-dose inhaler device will generally include a finely divided powder containing IL-17 homolog composition protein as a suspension in a non aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluroalkane-134a), HFA-227 (hydrofluroalkane-227), or the like. Preferably the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the IL-17 homolog composition protein as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases solution aerosols are preferred using solvents such as ethanol. Additional agents known in the art for formulation of a protein such as IL-17 homolog protein can also be included in the formulation.

One of ordinary skill in the art will recognize that the methods of the current invention may be achieved by pulmonary administration of IL-17 homolog compositions via devices not described herein.

Non-Pulmonary Alternative Delivery

The invention further relates to the administration of IL-17 homolog by oral, parenteral, subcutaneous, intramuscular, intravenous, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means. IL-17 homolog compositions may be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) administration particularly in the form of liquid solutions or suspensions; for use in vaginal or rectal administration particularly in semisolid forms such as creams and suppositories; for oral, buccal, or sublingual administration particularly in the form of tablets or capsules; or intranasally particularly in the form of powders, nasal drops or aerosols certain agents; or transdermally particularly in the form of a gel, ointment, lotion, suspension or patch delivery system with chemical enhancers such as dimethyl sulfoxide to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger, et al. In "Drug Permeation Enhancement"; Hsieh, D. S., Eds., pp. 59-90 (Marcel Dekker, Inc. New York 1994), or with oxidizing agents that enable the application of formulations containing proteins and peptides onto the skin (WO 98/53847), or applications of electric fields to create transient transport pathways such as electroporation, or to increase the mobility of charged drugs through the skin such as iontophoresis, or application of ultrasound such as sonophoresis (U.S. Pat. Nos. 4,309,989 and 4,767,402).

Formulations for oral rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the

intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and trasylol) to inhibit enzymatic degradation. The active constituent compound of the solid-type dosage form for oral administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, .alpha-tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc. Tablets and pills can be further processed into enteric coated preparations. The liquid preparations for oral administration include emulsion, syrup, elixir, suspension and solution preparations allowable for medical use. These preparations may contain inactive diluting agents ordinarily used in said field, e.g., water. Liposomes have also been described as drug delivery systems for insulin and heparin (U.S. Pat. No. 4,239,754). More recently, microspheres of artificial polymers of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals (U.S. Pat. No. 4,925,673). Furthermore, carrier compounds described in U.S. Pat. No. 5,879,681 and U.S. Pat. No. 5,5,871,753 are used to deliver biologically active agents orally are known in the art.

Formulations for parenteral administration may contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent,

according to known methods. Agents for injection may be a non-toxic, non-orally administrable diluting agent such as aqueous solution or a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an ordinary solvent, or suspending solvent, sterile involatile oil can be used. For these purposes, any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids; natural or synthetic or semisynthtetic mono- or di- or triglycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressured needle-less injection device as described in U.S. Pat. No. 5,851,198, and a laser perforator device as described in U.S. Pat. No. 5,839,446.

For absorption through mucosal surfaces, compositions and methods of administering IL-17 homolog include an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and an aqueous continuous phase, which promotes absorption through mucosal surfaces by achieving mucoadhesion of the emulsion particles (U.S. Pat. Nos. 5,514,670). Mucous surfaces suitable for application of the emulsions of the present invention may include corneal, conjunctival, buccal, sublingual, nasal, vaginal, pulmonary, stomachic, intestinal, and rectal routes of administration. Formulations for vaginal or rectal administration, e.g. suppositories, may contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for intranasal administration may be solid and contain as excipients, for example, lactose or may be aqueous or oily solutions of nasal drops. For buccal administration excipients include sugars, calcium stearate, magnesium stearate, pregelinatined starch, and the like (U.S. Pat. Nos. 5,849,695).

For transdermal administration, the drug is encapsulated in a delivery device such as a liposome or polymeric nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless otherwise stated). A number of suitable devices are known, including microparticles made of synthetic polymers such as polyhydroxy acids such as polylactic acid, polyglycolic acid and copolymers thereof, polyorthoesters, polyanhydrides, and polyphosphazenes, and natural polymers such as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof (U.S. Pat. Nos. 5,814,599).

It is often desirable to deliver the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year from a single administration. Various slow release, depot or implant dosage forms may be utilized. For example, a dosage form may contain a pharmaceutically acceptable non-toxic salt of the compounds which has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-dibenzyl-ethylenediamine or ethylenediamine; or (c) combinations of (a) and (b) e.g. a zinc tannate salt. Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt such as those just described, may be formulated in a gel, for example, an aluminum monostearate gel with, e.g. sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for

injection would contain the compound or salt dispersed for encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer for example as described in U.S. Pat. No. 3,773,919. The compounds or, preferably, relatively insoluble salts such as those described above may also be formulated in cholesterol matrix silastic pellets, particularly for use in animals. Additional slow release, depot or implant formulations, e.g. gas or liquid liposomes are known in the literature (U.S. Pat. Nos. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

ANTIGENIC/EPITOPE COMPRISING IL-17 PEPTIDE AND POLYPEPTIDES

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention according to methods well known in the art (See, e.g., Colligan et al,. ed., Current Protocols in Immunology, Greene Publishing, N.Y. (1993-1998), Ausubel, supra, entirely incorporated herein by reference).

The epitopes of the invention are immunogenic or antigenic epitopes of an IL-17 homolog polypeptide described herein. An "immunogenic epitope" can be defined as a part of a polypeptide that elicits an antibody response when the whole polypeptide is the immunogen. On the other hand, a region of a polypeptide molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a polypeptide generally is less than the number of antigenic epitopes (See, for instance, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998 (1983)).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a polypeptide molecule to which an antibody can bind), it is

well known in that art that relatively short synthetic peptides that mimic part of a polypeptide sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked polypeptide (See, for instance, Sutcliffe et al., Science 219:660 (1983)). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a polypeptide, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact polypeptides (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention (See, for instance, Wilson et al., Cell 37:767 (1984) at 777). Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

The epitope-bearing peptides and polypeptides of the invention can be produced by any conventional means (Houghten et al., Proc. Natl. Acad. Sci. USA 82:5131 (1985)). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten et al. (1986).

As one of skill in the art will appreciate, IL-17 homolog polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an

increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker et al., Nature 331:84 (1988)). Fusion proteins that have a disulfide-linked dimeric structure due to the IgG part can also be more efficient in binding other molecules than the monomeric IL-17 homolog polypeptide or polypeptide fragment alone(Fountoulakis et al., J. Biochem 270:3958 (1995)).

In one embodiment, the fusion is to the Fc region of an IgG molecule. The immunoglobulin constant region can contain genetic modifications including those which reduce or eliminate effector activity inherent in the immunoglobulin structure. (See, e.g., PCT Publication No. WO88/07089, published September 22, 1988). Briefly, PCR overlap extension is applied to join DNA encoding IL-17 homolog polypeptide to DNA encoding the hinge, CH2 and CH3 regions of human IgG1. The sequence of human IgG1 is obtained through Genbank (accession: HUMIGCC4; Takahashi et. al (vol.29, 671-679, 1982). This is compiled into exons and a region upstream of the natural hinge region is chosen as the fusion site.

The nucleotide sequence of the polypeptide-IgG gene fusions can be modified to replace cysteine residues in the hinge region with serine residues and/or amino acids within the CH2 domain which are believed to be required for IgG binding to Fc receptors and complement activation.

Alternatively, Fc fusion proteins can be generated using an expression vector such as the CD5-IgG1 vector (described by Aruffo et al., (1990), Cell, 61:1303-1313), which already contains the IgG constant region. According to this method, the DNA fragment encoding the IL-17 homolog

polypeptide is generated in a PCR reaction so that the open reading frame will be continuous and in frame with that encoding the IgG constant region when the resulting PCR fragment is cloned into the CD5-IgG vector (Aruffo et al., (1990), Cell).

PRODUCTION OF ANTIBODIES

The polypeptides of this invention and fragments thereof may be used in the production of antibodies. The term "antibody" as used herein describes antibodies, fragments of antibodies (such as, but not limited, to Fab, Fab', Fab2', and Fv fragments), and modified versions thereof, as well known in the art (e.g., chimeric, humanized, recombinant, veneered, resurfaced or CDR-grafted) such antibodies are capable of binding antigens of a similar nature as the parent antibody molecule from which they are derived. The instant invention also encompasses single chain polypeptide binding molecules.

The production of antibodies, both monoclonal and polyclonal, in animals is well known in the art (See, e.g., Colligan, supra).

Single chain antibodies and libraries thereof are yet another variety of genetically engineered antibody technology that is well known in the art (See, e.g. R.E. Bird et al., Science 242:423 (1988); PCT Publication Nos. WO 88/01649, WO 90/14430, and WO 91/10737). Single chain antibody technology involves covalently joining the binding regions of heavy and light chains to generate a single polypeptide chain. The binding specificity of the intact antibody molecule is thereby reproduced on a single polypeptide chain.

Antibodies included in this invention are useful in diagnostics, therapeutics or in diagnostic/therapeutic combinations.

The polypeptides of this invention or suitable fragments thereof can be used to generate polyclonal or monoclonal antibodies, and various inter-species hybrids, or humanized antibodies, or antibody fragments, or single-chain antibodies. The techniques for producing antibodies are well known to skilled artisans. (See, e.g., Colligan supra; Monoclonal Antibodies: Principles & Applications Ed. J.R.Birch & E.S. Lennox, Wiley-Liss, 1995).

A polypeptide used as an immunogen may be modified or administered in an adjuvant, by subcutaneous or intraperitoneal injection into, for example, a mouse or a rabbit. For the production of monoclonal antibodies, spleen cells from immunized animals are removed, fused with myeloma or other suitable known cells, and allowed to become monoclonal antibody producing hybridoma cells in the manner known to the skilled artisan. Hybridomas that secrete a desired antibody molecule can be screened by a variety of well known methods, for example ELISA assay, Western blot analysis, or radioimmunoassay (Lutz et al., Exp. Cell Res. 175:109 (1988); Monoclonal Antibodies: Principles & Applications Ed. J.R.Birch & E.S. Lennox, Wiley-Liss, 1995; Colligan, supra).

For some applications labeled antibodies are desirable. Procedures for labeling antibody molecules are widely known, including for example, the use of radioisotopes, affinity labels, such as biotin or avidin, enzymatic labels, for example horseradish peroxidase, and fluorescent labels, such as FITC or rhodamine (See, e.g., Colligan, supra).

Labeled antibodies are useful for a variety of diagnostic applications. In one embodiment the present

invention relates to the use of labeled antibodies to detect the presence of an IL-17 homolog polypeptide. Alternatively, the antibodies could be used in a screen to identify potential modulators of an IL-17 homolog polypeptide. For example, in a competitive displacement assay, the antibody or compound to be tested is labeled by any suitable method. Competitive displacement of an antibody from an antibody-antigen complex by a test compound such that a test compound-antigen complex is formed provides a method for identifying compounds that bind HPLFP.

TRANSGENICS AND CHIMERIC NON-HUMAN ANIMALS

The present invention is also directed to a transgenic non-human eukaryotic animal (preferably a rodent, such as a mouse) the germ cells and somatic cells of which contain genomic DNA according to the present invention which codes for an IL-17 homolog polypeptide. At least one nucleic acid encoding an IL-17 homolog polypeptide can be introduced into the animal to be made transgenic, or an ancestor of the animal, at an embryonic stage, preferably the 1-1000 cell or oocyte, stage, and preferably not later than about the 64-cell stage. The term "transgene," as used herein, means a gene which is incorporated into the genome of the animal and is expressed in the animal, resulting in the presence of at least one IL-17 homolog polypeptide in the transgenic animal. Such animals are preferably non-human mammals.

There are several means by which such nucleic acid encoding an IL-17 homolog can be introduced into a cell or genome of the animal embryo so as to be chromosomally incorporated and expressed according to known methods.

Chimeric non-human animals, and preferably mammals, in which fewer than all of the somatic and germ cells contain the an IL-17 homolog polypeptide encoding nucleic acid of

the present invention, such as animals produced when fewer than all of the cells of the morula are transfected in the process of producing the transgenic animal, are also intended to be within the scope of the present invention.

Chimeric non-human mammals having human cells or tissue engrafted therein are also encompassed by the present invention, which may be used for testing expression of at least one IL-17 homolog polypeptide in human tissue and/or for testing the effectiveness of therapeutic and/or diagnostic agents associated with delivery vectors which preferentially bind to an IL-17 homolog polypeptide of the present invention. Methods for providing chimeric non-human mammals are provided, e.g, in U.S. serial Nos. 07/508,225, 07/518,748, 07/529,217, 07/562,746, 07/596,518, 07/574,748, 07/575,962, 07/207,273, 07/241,590 and 07/137,173.

The techniques described in Leder, U.S. Patent 4,736,866 for producing transgenic non-human mammals may be used for the production of a transgenic non-human mammal of the present invention. The various techniques described in Palmiter et al., Ann. Rev. Genet. 20:465 (1986), the entire contents of which are hereby incorporated by reference, may also be used.

The animals carrying at least one IL-17 homolog polypeptide nucleic acid can be used to test compounds or other treatment modalities which may prevent, suppress or cure a pathology using an IL-17 homolog polypeptide or IL-17 nucleic acid of the present invention. Such transgenic animals will also serve as a model for testing of diagnostic methods for the same diseases. Transgenic animals according to the present invention can also be used as a source of cells for cell culture. Transgenic or chimeric non-human animals according to the present invention can also be used as a source of IL-17 homolog polypeptide. For example, such

an animal is designed so that IL-17 homolog polypeptide is expressed in one or more tissues of the animal and the polypeptide is recovered from these tissues by methods known to the skilled artisan.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

EXAMPLE 1: EXPRESSION AND PURIFICATION OF AN IL-17 HOMOLOG POLYPEPTIDE IN E. COLI

The bacterial expression vector pQE60 is used for bacterial expression in this example. (QIAGEN, Inc., Chatsworth, CA). pQE60 encodes ampicillin antibiotic resistance ("Ampr") and contains a bacterial origin of replication ("ori"), an IPTG inducible promoter, a ribosome binding site ("RBS"), six codons encoding histidine residues that allow affinity purification using nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin sold by QIAGEN, Inc., and suitable single restriction enzyme cleavage sites. These elements are arranged such that a DNA fragment encoding a polypeptide can be inserted in such as way as to produce that polypeptide with the six His residues (i.e., a "6 X His tag") covalently linked to the carboxyl terminus of that polypeptide. However, a polypeptide coding sequence can optionally be inserted such that translation of the six His codons is prevented and, therefore, a polypeptide is produced with no 6 X His tag.

The nucleic acid sequence encoding the desired portion of an IL-17 homolog polypeptide lacking the hydrophobic leader sequence is amplified from a cDNA clone using PCR oligonucleotide primers (based on the sequences presented,

(e.g., as presented in SEQ ID NO:1), which anneal to the amino terminal sequences of the desired portion of a nucleotide sequence encoding an IL-17 homolog polypeptide and to sequences in a construct 3′ to the cDNA coding sequence. Additional nucleotides containing restriction sites to facilitate cloning in the pQE60 vector are added to the 5′ and 3′ sequences, respectively.

For cloning an IL-17 homolog polypeptide, the 5′ and 3′ primers have nucleotides corresponding or complementary to a portion of the coding sequence of a nucleic acid encoding IL-17 homolog polypeptide, e.g., as presented in SEQ ID NO:1, according to known method steps. One of ordinary skill in the art would appreciate, of course, that the point in a polypeptide coding sequence where the 5′ primer begins can be varied to amplify a desired portion of the complete polypeptide shorter or longer than the mature form.

The amplified IL-17 homolog nucleic acid fragments and the vector pQE60 are digested with appropriate restriction enzymes and the digested DNAs are then ligated together. Insertion of the IL-17 homolog encoding DNA into the restricted pQE60 vector places an IL-17 homolog polypeptide coding region including its associated stop codon downstream from the IPTG-inducible promoter and in-frame with an initiating AUG. The associated stop codon prevents translation of the six histidine codons downstream of the insertion point.

The ligation mixture is transformed into competent E. coli cells using standard procedures such as those described in Sambrook et al.,1989; Ausubel, 1987-1998. E. coli strain M15/rep4, containing multiple copies of the plasmid pREP4, which expresses the lac repressor and confers kanamycin resistance ("Kanr"), is used in carrying out the illustrative example described herein. This strain, which

is only one of many that are suitable for expressing IL-17 homolog polypeptide, is available commercially from QIAGEN, Inc. Transformants are identified by their ability to grow on LB plates in the presence of ampicillin and kanamycin. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Clones containing the desired constructs are grown overnight ("O/N") in liquid culture in LB media supplemented with both ampicillin (100 mg/ml) and kanamycin (25 mg/ml). The O/N culture is used to inoculate a large culture, at a dilution of approximately 1:25 to 1:250. The cells are grown to an optical density at 600 nm ("OD600") of between 0.4 and 0.6. Isopropyl-b-D-thiogalactopyranoside ("IPTG") is then added to a final concentration of 1 mM to induce transcription from the lac repressor sensitive promoter, by inactivating the lacI repressor. Cells subsequently are incubated further for 3 to 4 hours. Cells then are harvested by centrifugation.

The cells are then stirred for 3-4 hours at 4°C in 6M guanidine-HCl, pH8. The cell debris is removed by centrifugation, and the supernatant containing the IL-17 is dialyzed against 50 mM Na-acetate buffer pH6, supplemented with 200 mM NaCl. Alternatively, a polypeptide can be successfully refolded by dialyzing it against 500 mM NaCl, 20% glycerol, 25 mM Tris/HCl pH 7.4, containing protease inhibitors. After renaturation the polypeptide is purified by ion exchange, hydrophobic interaction and size exclusion chromatography. Alternatively, an affinity chromatography step such as an antibody column is used to obtain pure IL-17 homolog polypeptide. The purified polypeptide is stored at 4°C or frozen at -80°C.

EXAMPLE 2: CLONING AND EXPRESSION OF AN IL-17 HOMOLOG POLYPEPTIDE IN A BACULOVIRUS EXPRESSION SYSTEM

In this illustrative example, the plasmid shuttle vector pA2 GP is used to insert the cloned DNA encoding the mature polypeptide into a baculovirus to express an IL-17 homolog polypeptide, using a baculovirus leader and standard methods as described in Summers et al., A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures, Texas Agricultural Experimental Station Bulletin No. 1555 (1987). This expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by the secretory signal peptide (leader) of the baculovirus gp67 polypeptide and convenient restriction sites such as BamHI, XbaI and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from E. coli under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate viable virus that expresses the cloned polynucleotide.

Other baculovirus vectors are used in place of the vector above, such as pAc373, pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31.

The cDNA sequence encoding the mature IL-17 homolog polypeptide in a clone, lacking the AUG initiation codon and the naturally associated nucleotide binding site, is amplified using PCR oligonucleotide primers corresponding to the 5′ and 3′ sequences of the gene. Non-limiting examples include 5′ and 3′ primers having nucleotides corresponding or complementary to a portion of the coding sequence of a nucleic acid encoding an IL-17 homolog , e.g., as presented in SEQ ID NO:1, according to known method steps.

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit (e.g., "Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is then digested with the appropriate restriction enzyme and again is purified on a 1% agarose gel. This fragment is designated herein "F1."

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.). This vector DNA is designated herein "V1."

Fragment F1 and the dephosphorylated plasmid V1 are ligated together with T4 DNA ligase. E. coli HB101 or other suitable E. coli hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria are identified that contain the plasmid with the human IL-17 gene using the PCR method, in which one of the primers that is used to amplify the gene and the second primer is from well within the vector so that only those bacterial colonies containing the IL-17 homolog gene fragment will show amplification of the DNA. The sequence of the cloned

fragment is confirmed by DNA sequencing. This plasmid is designated herein pBac IL-17.

Five mg of the plasmid pBacIL-17 is co-transfected with 1.0 mg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA.), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413 (1987). 1 mg of BaculoGold™ virus DNA and 5 mg of the plasmid pBacIL-17 are mixed in a sterile well of a microtiter plate containing 50 ml of serum-free Grace's medium (Life Technologies Inc., Rockville, MD). Afterwards, 10 ml Lipofectin plus 90 ml Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is rocked back and forth to mix the newly added solution. The plate is then incubated for 5 hours at 27°C. After 5 hours the transfection solution is removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. The plate is put back into an incubator and cultivation is continued at 27°C for four days.

After four days the supernatant is collected and a plaque assay is performed, according to known methods. An agarose gel with "Blue Gal" (Life Technologies Inc., Rockville, MD) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Rockville, MD, page 9-10). After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar

containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ml of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4°C. The recombinant virus is called V-IL-17.

To verify the expression of the IL-17 homolog gene, Sf9 cells are grown in Grace's medium supplemented with 10% heat inactivated FBS. The cells are infected with the recombinant baculovirus V-IL-17 at a multiplicity of infection ("MOI") of about 2. Six hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). If radiolabeled polypeptides are desired, 42 hours later, 5 mCi of 35S-methionine and 5 mCi 35S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then they are harvested by centrifugation. The polypeptides in the supernatant as well as the intracellular polypeptides are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled). Microsequencing of the amino acid sequence of the amino terminus of purified polypeptide can be used to determine the amino terminal sequence of the mature polypeptide and thus the cleavage point and length of the secretory signal peptide.

EXAMPLE 3: CLONING AND EXPRESSION OF IL-17 HOMOLOG POLYPEPTIDE IN MAMMALIAN CELLS

A typical mammalian expression vector contains the promoter element, which mediates the initiation of transcription of mRNA, the polypeptide coding sequence, and signals required for the termination of transcription and

polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pIRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clonetech Labs, Palo Alto, CA), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include human Hela 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells. Preferred cells are HEK293 and AV12 cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded polypeptide. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS). Murphy et al., Biochem J. 227:277 (1991); Bebbington et al., Bio/Technology 10:169 (1992). Using these markers, the mammalian cells are

grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of polypeptides.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molec. Cell. Biol. 5:438 (1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

The expression plasmid, pIL-17 HA, is made by cloning a cDNA encoding IL-17 homolog into the expression vector pcDNAI/Amp or pcDNAIII (which can be obtained from Invitrogen, Inc.).

The expression vector pcDNAI/amp contains: (1) an E. coli origin of replication effective for propagation in E. coli and other prokaryotic cells; (2) an ampicillin resistance gene for selection of plasmid-containing prokaryotic cells; (3) an SV40 origin of replication for propagation in eucaryotic cells; (4) a CMV promoter, a polylinker, an SV40 intron; (5) several codons encoding a hemagglutinin fragment (i.e., an "HA" tag to facilitate purification) followed by a termination codon and polyadenylation signal arranged so that a cDNA can be conveniently placed under expression control of the CMV promoter and operably linked to the SV40 intron and the polyadenylation signal by means of restriction sites in the polylinker. The HA tag corresponds to an epitope derived from the influenza hemagglutinin polypeptide described by

Wilson et al., Cell 37:767 (1984). The fusion of the HA tag to the target polypeptide allows easy detection and recovery of the recombinant polypeptide with an antibody that recognizes the HA epitope. pcDNAIII contains, in addition, the selectable neomycin marker.

A DNA fragment encoding the IL-17 homolog is cloned into the polylinker region of the vector so that recombinant polypeptide expression is directed by the CMV promoter. The plasmid construction strategy is as follows. cDNA encoding IL-17 homolog polypeptide is amplified using primers that contain convenient restriction sites, much as described above for construction of vectors for expression of IL-17 homolog in E. coli. Non-limiting examples of suitable primers include those based on the coding sequences presented in SEQ ID NO:1, as they encode IL-17 homolog polypeptides as described herein.

The PCR amplified DNA fragment and the vector, pcDNAI/Amp, are digested with suitable restriction enzyme(s) and then ligated. The ligation mixture is transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037), and the transformed culture is plated on ampicillin media plates which then are incubated to allow growth of ampicillin resistant colonies. Plasmid DNA is isolated from resistant colonies and examined by restriction analysis or other means for the presence of the IL-17 homolog -encoding fragment.

For expression of recombinant IL-17 homolog , COS cells are transfected with an expression vector, as described above, using DEAE-DEXTRAN, as described, for instance, in Sambrook et al., Molecular Cloning: a Laboratory Manual, Cold Spring Laboratory Press, Cold Spring Harbor, New York

(1989). Cells are incubated under conditions for expression of IL-17 by the vector.

Expression of the IL-17-HA fusion polypeptide is detected by radiolabeling and immunoprecipitation, using methods described in, for example Harlow et al., Antibodies: A Laboratory Manual, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1988). To this end, two days after transfection, the cells are labeled by incubation in media containing 35S-cysteine for 8 hours. The cells and the media are collected, and the cells are washed and lysed with detergent-containing RIPA buffer: 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM TRIS, pH 7.5, as described by Wilson et al. cited above. Proteins are precipitated from the cell lysate and from the culture media using an HA-specific monoclonal antibody. The precipitated polypeptides then are analyzed by SDS-PAGE and autoradiography. An expression product of the expected size is seen in the cell lysate, which is not seen in negative controls.

The vector pC4 is used for the expression of IL-17 homolog polypeptide. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (alpha minus MEM, Life Technologies) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., Alt et al., J Biol. Chem. 253:1357 (1978), Hamlin et al., Biochem. et Biophys. Acta, 1097:107 (1990), Page et al., Biotechnology 9:64 (1991)). Cells grown in increasing

concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained which contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen et al., Molec. Cell. Biol. 5:438 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart et al., Cell 41:521 (1985)). Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human b-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the IL-17 in a regulated way in mammalian cells. Gossen et al., Proc. Natl. Acad. Sci. USA 89:5547 (1992). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous

to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete IL-17 homolog polypeptide including its nucleotide binding site is amplified using PCR oligonucleotide primers corresponding to the 5′ and 3′ sequences of the gene. Non-limiting examples include 5′ and 3′ primers having nucleotides corresponding or complementary to a portion of the coding sequence of an IL-17 homolog encoding nucleic acid, e.g., as presented in SEQ ID NO:1, according to known method steps.

The amplified fragment is digested with suitable endonucleases and then purified again on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. E. coli HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. 5 mg of the expression plasmid pC4 is cotransfected with 0.5 mg of the plasmid pSV2-neo using lipofectin. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well

petri dishes or 10ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

EXAMPLE 4 PURIFICATION OF MATURE IL-17H

Media containing mature IL-17h cloned and expressed according to standard techniques was initially filtered through a glass fiber filter; then loaded onto a chelate column (Qiagen Ni - NTA Superflow) at 40mls/min. The column was then washed with 1x PBS pH=7.4 until the A280nm returned to baseline. A gradient elution with imidazole (approx. 1.3%/min.) was performed, at a flow rate of 8mls/min, to elute the bound IL-17h. The fractions containing IL-17h were pooled and dialyzed in 6-8,000 MWCO tubing against 1x PBS pH=7.4 at 4°C for at least 4 hours. The buffer was then exchanged at least once with fresh 1x PBS pH=7.4. The total dialysis was no less than 1/100,000 fold. The dialyzed protein pool was then filtered through a .2 micron sterile Gelman Acrodisc into a sterile vial. Alternatively, the bound protein was eluted from the Ni-NTA column using a pH gradient from pH=7.4 to pH=4.0. Fractions containing IL-17h were neutralized to pH=7.4 with 0.5M sodium phosphate buffer (pH=9.25). The protein pool was then filtered through a .2 micron sterile Gelman Acrodisc into a sterile vile.

EXAMPLE 5: TISSUE DISTRIBUTION OF IL-17 mRNA EXPRESSION

Using standard techniques, RT-PCR analysis was performed on several commercially available cDNA libraries (Clontech). IL-17 homolog polypeptide encoding nucleic acid was selectively detected in leukocytes, fetal liver and testis.

EXAMPLE 6: GENERATION OF MICE CONTAINING HEMATOPOEITIC CELLS TRANSDUCED WITH DNA ENCODING IL-17H

Phoenix-E cells and the Lazarus vector were obtained from G. Nolan (Stanford University). A cDNA encoding SEQ ID NO:2 was cloned into the BamHI and EcoRI sites of pLZRS/IB (a derivative of the Lazarus vector containing an IRES (internal ribosome entry site) blasticidin cassette for blasticidin drug resistance). The DNA was transfected into Phoenix-E cells with Lipofectamine Plus (Gibco/BRL, Gaithersburg, MD) according to the manufacturer's instructions. The transfected cells were selected in puromycin (Calbiochem, La Jolla, CA) for 7 days, expanded into large tissue culture flasks and virus was harvested from the cells at 32^{0}C. The virus was filtered, aliquoted, and stored at -80^{0}C. Virus was titered on 3T6 cells (ATCC) and was determined to be greater than 10^6 cfu/ml.

For generation of retroviral mice, Balb/c male mice (Harlan Sprague Dawley, Indianapolis, IN) were injected i.p. with 5-Fluorouracil (Calbiochem, La Jolla, CA) at 150 mg/kg body weight. Four days later, the mice were euthanised and bone marrow cells were isolated by flushing out femurs with a syringe filled with media and a 23G needle. 2 x 10^6 cells were seeded into wells of a 24 well tray and stimulated for 2 days in Iscoves modified Dulbeccos media (IMDM) (Gibco/BRL, Gaithersburg, MD) containing 30% FBS (Stem Cell Technolgies, Vancouver, CA), 1% Pen/Strep (Gibco/BRL, Gaithersburg, MD), 100 U/ml IL-3, 100 ng/ml SCF, and 100

ng/ml IL-6. All cytokines were purchased from R&D Systems (Minneapolis, MN).

Following stimulation, cells were transduced with 4 mls of retroviral supernatant in the same media on fibronectin coated plates (Panvera). Retroviral transduction was repeated after 4 hours and the cells were then cultured for 24 hours. Cells were then placed into the same media with blasticidin at 10 g/ml (Calbiochem, La Jolla, CA) and selected for 3 days. Following selection in blasticidin, 1 x 10^6 cells were injected via tail vein into lethally irradiated male Balb/c mice (split dose of 550 rads, 3 hours apart). Control mice received vector alone transduced bone marrow cells. Some transduced bone marrow cells were also plated out for a CFU (colony forming unit) assay as follows: 2 x 10^4 cells were plated out in triplicate in dishes containing complete methylcellulose media with cytokines (Stem Cell Technologies, Vancouver, CA). Some dishes also contained blasticidin at 10 g/ml. After 7 days, colonies were scored on an inverted microscope according to colony size and morphology.

Mice were analyzed 18, 24, 40, and 120 days post-transplant for changes in blood chemistry, hematology, bone marrow progenitor content, and histopathology of hematopoeitic tissues and compared to control animals. The IL-17 animals generally looked unhealthy and exhibited an average of ten percent decrease in body weight. Mouse serum was analyzed for IL-17 homolog polypeptide FLAG epitope tagged protein by Western blot analysis. The following data in Table 1 was generated using method steps as known in the art and as described herein:

TABLE 1: Analysis of Retroviral Mice at 18 days Post-transplant

Mouse	BFU-E/CFU-E	CFU-GM	CFU-GEMM	CFC total	% of 5 x 10^4 cells plated	%Blast. Res.
IB#1	6	30	0	36	0.072	25
IB#2	4	38	1	43	0.086	12
IB#3	3	29	0	32	0.064	31
Avg.	**4**	**32**	**0.33**	**37**	**0.074**	**23**
IL17h#1	8	79	1	88	0.176	69
IL17h#2	1	28	1	30	0.060	76
IL17h#3	7	83	0	90	0.180	38
IL17h#4	5	84	1	90	0.180	82
Avg.	**5**	**69**	**0.75**	**75**	**0.15**	**66**

Mouse	Weight (*gms)	BM cells/femur (x 10^7)	CFC/femur	Spleen Cellularity/mg (x 10^5)
IB#1	23.5 (+0.6)	0.242	1742	8.45
IB#2	23.4 (+0.4)	0.389	3345	5.06
IB#3	20.1 (+0.3)	0.682	4365	7.55
Avg.	**22.33**	**0.438**	**3151**	**7.02**
IL17h				
IL17h#1	26.1 (+2.0)	0.968	17037	6.48
IL17h#2	25.4 (-0.3)	0.55	3300	15.3
IL17h#3	26.1 (-1.3)	0.92	16560	6.45
IL17h#4	22.1 (+1.5)	0.264	4752	9.46
Avg.	**24.9**	**0.676**	**10412**	**9.42**

* Number in parentheses indicates the change in body weight compared to the weight of the mouse the day before transplantation.

In Table 1, IB 1-3 are control animals and the abbreviations are defined as: CFU-E: colony-forming unit-erythroid; CFU-GM: colony-forming unit-granulocyte, macrophage; CFU-GEMM: colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte; CFC: colony-forming cell; BFU-E: burst-forming unit-erythroid. These data show that cells retrovirally transduced with DNA encoding IL-17h increase, or mediate an increase in, bone marrow and spleen cellularity, eosinophils, and Colony Forming Cells (CFCs)

and decrease lymphocytes, as compared to controls. IL-17h thus modulates, e.g., stimulates, hematopoietic cell growth, including the stimulation of proliferation and/or differentiation of at least one early or multipotent progenitor committed to at least one granulocyte-macrophage and/or megakaryocyte lineage.

Furthermore, the effects of IL-17h in in vitro colony assays was confirmed by blood chemistry analysis of retroviral mice expressing IL-17h. As shown in Figures 1 and 2, mice transplanted with IL-17h-expressing bone marrow cells had increased counts of neutrophils, white blood cells and eosinophils (Figure 1), as well as platelets (Figure 2). These data show that IL-17h accelerates the recovery of peripheral blood leukocytes, especially neutrophils and platelets, following lethal irradiation and bone marrow transplantation.

EXAMPLE 7: EFFECTS OF MATURE IL-17H ON COLONY FORMATION IN HUMAN AND MOUSE BONE MARROW

As shown in Figure 3, mature IL-17h increased colony formation of human bone marrow cells in semisolid cultures when such cells were treated with mature IL-17h plus EPO, GM-CSF, IL-3, and stem cell factor (SCF). Figure 3 shows increased BFR-E, CFU-GEMM, and CFU-GM colonies for the mature IL-17h (in the presence of erythropoietin (3U/ml), IL-3 (10ng/ml), and stem cell factor (50ng/ml))treated group as compared to the control (abbreviations are defined in the brief description of Figure 3). The human bone marrow cells were purchased from Poietic Technologies (Gaithersburg, MD) and the reagents were bought from Stem Cell Technologies, Inc. (Vancouver, Canada). The assays were performed using routine techniques as described in Current Protocols in Immunology and the procedure manual from Stem Cell

Technologies.

Figure 4 shows that mature IL-17h potentiated activity of stem cell factor on colony formation of murine bone marrow granulocyte, macrophage cells. The murine bone marrow cells were obtained from femurs of BALB/C mice two days following 5-fluorouracil injection. 2.5X104 bone marrow cells were plated out in triplicate with stem cell factor or stem cell factor plus mature IL-17h in methylcellulose media. 7 days later, total colony forming units were scored. Mature IL-17h increased colony-forming units as compared to stem cell factor alone.

EXAMPLE 8: EFFECTS OF MATURE IL-17H ON ICAM-1 EXPRESSION

Human dermal fibroblasts from Clonetics were plated at $2x10^5$ cells /well in a 6-well dish with mature IL-17h in 2ml Fibroblast Basal Medium (Clonetics) + 2% heat inactivated FBS. Cells were incubated for 48 hours, trypsinized in low trypsin (Clonetics), spun down, and resuspended in 50µl 0.1% BSA/PBS. The cells were then stained for 30 minutes at 4°C with either monoclonal anti-human ICAM-1-Fluorescein (R&D Systems BBA20) or anti-mouse IgG1-Fluorescien (isotype control). After washing with 0.1%BSA/PBS, the cells were analyzed by flow cytometric analysis.

As shown in Figure 5, mature IL-17h protein upregulates ICAM-1 on Normal Human Dermal Fibroblasts. IL-17 and other cytokines similarly upregulate ICAM-1 which is an important initial step in cell to cell communication leading to extravasation of leukocytes, for example, into to other surrounding tissues.

EXAMPLE 9: EFFECTS OF MATURE IL-17H ON IL-6 SECRETION

Human dermal fibroblasts from Clonetics were plated at $2x10^5$ cells /well in a 6-well dish with mature IL-17h in 2ml

Fibroblast Basal Medium (Clonetics) + 2% heat inactivated FBS. Cells were incubated for 48 hours, after which 100µl cell free supernatant was assayed for IL-6 using the R&D Systems human IL-6 Quantikine.

As shown in Figure 6, mature IL-17h protein increases IL-6 secretion from Normal Human Dermal Fibroblasts. IL-17 and other cytokines similarly upregulate IL-6 secretion.

EXAMPLE 10: EFFECTS OF MATURE IL-17H ON IL-8 SECRETION

Human dermal fibroblasts from Clonetics were plated at $2x10^5$ cells /well in a 6-well dish with mature IL-17h in 2ml Fibroblast Basal Medium (Clonetics) + 2% heat inactivated FBS. Cells were incubated for 48 hours, after which 100ml cell free supernatant was assayed for IL-6 using the R&D Systems human IL-8 Quantikine.

As shown in Figure 7, mature IL-17h protein increases IL-8 secretion from Normal Human Dermal Fibroblasts as does IL-17 and other cytokines.

It will be clear that the invention can be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

Priority United States Provisional Patent Application Serial Nos: 60/102,883 (filed October 2, 1998), 60/110,405 (filed December 1, 1998), and 60/138,910 (filed June 11, 1999) are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

<110> Glasebrook, Andrew L.
Su, Eric
Wei, Jian-Jun
Liu, Ling

<120> Human IL-17 Homolog Nucleic Acids, Polypeptides, Vectors, Host Cells, Methods and Uses Thereof

<130> X-12190PCT

<160> 3

<170> PatentIn Ver. 2.0

<210> 1
<211> 591
<212> DNA
<213> Homo sapiens

<400> 1
atgacgctcc tccccggcct cctgtttctg acctggctgc acacatgcct ggcccaccat 60
gacccctccc tcaggggggca cccccacagt cacggtaccc cacactgcta ctcggctgag 120
gaactgcccc tcggccaggc ccccccacac ctgctggctc gaggtgccaa gtgggggcag 180
gctttgcctg tagccctggt gtccagcctg gaggcagcaa gccacagggg gaggcacgag 240
aggccctcag ctacgaccca gtgcccggtg ctgcggccgg aggaggtgtt ggaggcagac 300
acccaccagc gctccatctc accctggaga taccgtgtgg acacggatga ggaccgctat 360
ccacagaagc tggccttcgc cgagtgcctg tgcagaggct gtatcgatgc acggacgggc 420
cgcgagacag ctgcgctcaa ctccgtgcgg ctgctccaga gcctgctggt gctgcgccgc 480
cggccctgct cccgcgacgg ctcggggctc cccacacctg gggcctttgc cttccacacc 540
gagttcatcc acgtccccgt cggctgcacc tgcgtgctgc cccgttcagt g 591

<210> 2
<211> 197
<212> PRT
<213> Homo sapiens

<400> 1
Met Thr Leu Leu Pro Gly Leu Leu Phe Leu Thr Trp Leu His Thr Cys
1 5 10 15

Leu Ala His His Asp Pro Ser Leu Arg Gly His Pro His Ser His Gly
20 25 30

Thr Pro His Cys Tyr Ser Ala Glu Glu Leu Pro Leu Gly Gln Ala Pro
35 40 45

Pro His Leu Leu Ala Arg Gly Ala Lys Trp Gly Gln Ala Leu Pro Val
50 55 60

Ala Leu Val Ser Ser Leu Glu Ala Ala Ser His Arg Gly Arg His Glu
65 70 75 80

Arg Pro Ser Ala Thr Thr Gln Cys Pro Val Leu Arg Pro Glu Glu Val
85 90 95

Leu Glu Ala Asp Thr His Gln Arg Ser Ile Ser Pro Trp Arg Tyr Arg

```
                100                 105                 110

Val Asp Thr Asp Glu Asp Arg Tyr Pro Gln Lys Leu Ala Phe Ala Glu
        115                 120                 125

Cys Leu Cys Arg Gly Cys Ile Asp Ala Arg Thr Gly Arg Glu Thr Ala
    130                 135                 140

Ala Leu Asn Ser Val Arg Leu Leu Gln Ser Leu Leu Val Leu Arg Arg
145                 150                 155                 160

Arg Pro Cys Ser Arg Asp Gly Ser Gly Leu Pro Thr Pro Gly Ala Phe
                165                 170                 175

Ala Phe His Thr Glu Phe Ile His Val Pro Val Gly Cys Thr Cys Val
            180                 185                 190

Leu Pro Arg Ser Val
        195
```

WE CLAIM:

1. An isolated nucleic acid comprising a polynucleotide encoding an IL-17 homolog polypeptide.

2. The isolated nucleic acid of claim 1, comprising a polynucleotide that hybridizes under stringent conditions to a nucleic acid encoding amino acids 19-197 of SEQ ID NO:2 or the complement thereof.

3. The isolated nucleic acid of claim 1, comprising a polynucleotide that hybridizes under stringent conditions to SEQ ID:1 or the complement thereof.

4. The isolated nucleic acid of claim 1, comprising a polynucleotide that encodes the polypeptide of SEQ ID NO:2.

5. The isolated nucleic acid of claim 1, comprising a polynucleotide that encodes amino acids 19-197 of SEQ ID NO:2.

6. The isolated nucleic acid of claim 1, comprising the nucleic acid sequence of SEQ ID NO:1.

7. The isolated nucleic acid of claim 1, comprising nucleotides 55-591 of SEQ ID NO:1.

8. A method for obtaining the isolated nucleic acid of claim 1, comprising (1)contacting under stringent hybridization conditions a first nucleic acid sequence with one or more additional nucleic acid sequences selected from a suitable source, wherein said first nucleic acid sequence comprises a polynucleotide encoding amino acids 19-197 of SEQ ID NO:2 or the complement thereof, and (2) isolating from said one or more sequences at least one sequence that has hybridized to said first nucleic acid sequence.

9. An isolated nucleic acid, comprising a nucleic acid sequence obtainable by the method of claim 8.

10. A composition, comprising an isolated nucleic acid of claim 1 and a carrier or diluent.

11. A vector, comprising the nucleic acid according claim 1.

12. A host cell comprising the isolated nucleic acid according to claim 1.

13. An isolated IL-17 homolog polypeptide.

14. The isolated polypeptide of claim 13, comprising a polypeptide having at least 80% sequence identity to amino acids 19-197 of SEQ ID NO:2.

15. The isolated polypeptide of claim 13, wherein said polypeptide comprises amino acids 19-197 of SEQ ID NO:2.

16. The isolated polypeptide of claim 13, wherein said polypeptide comprises the amino acid sequence of SEQ ID NO:2.

17. A composition, comprising the isolated polypeptide of claim 13 and a carrier or diluent.

18. An isolated antibody or at least one fragment thereof that binds an epitope specific to a polypeptide according to claim 13.

19. A host cell expressing at least one antibody or at least one fragment thereof according to claim 18.

20. A method for producing at least one antibody, comprising culturing the host cell according to claim 19.

21. A method for producing at least one IL-17 homolog polypeptide, comprising translating the nucleic acid of claim 1 under conditions that the IL-17 homolog polypeptide is expressed in detectable or recoverable amounts.

22. An isolated IL-17 homolog polypeptide obtainable by the method of claim 21.

23. A transgenic or chimeric non-human animal, comprising at least one host cell according to claim 12.

24. The composition of claim 17, wherein said carrier or diluent is pharmaceutically acceptable.

25. The composition of claim 17, further comprising at least one compound or protein having at least one activity selected from a hematopoietic activity, a erythropoietic activity, a leukopoietic activity, a thrombopoietic activity, an IL-6 activity, an IL-8 activity, an ICAM-1 activity, an IL-11 activity, an IL-17 activity, or a TPO activity.

26. The composition of claim 17, further comprising at least one compound or protein selected from an erythropoietin, an interleukin-3, a colony stimulating factor (CSF), a granulocyte-macrophage colony stimulating factor (GM-CSF), a stem cell factor (SCF), an interleukin-6, an interleukin-8, an interleukin-11, or an interleukin-17.

27. A method for stimulating hematopoiesis in a cell, tissue, organ or animal, comprising contacting or administering an effective amount of at least one IL-17 homolog polypeptide according to claim 13 with or to said cell, tissue, organ or animal.

28. The method of claim 27, wherein said animal is a primate.

29. The method of claim 28, wherein said primate is a monkey or a human.

30. The method of claim 27, wherein said hematopoiesis is at least one selected from erythropoiesis, leukopoiesis, and thrombocytopoiesis.

31. The method of claim 27, wherein said effective amount is 0.001-10 mg/kilogram of said cells, tissue, organ or animal.

32. A method for stimulting production of neutrophils, granulocytes, or megakaryocytes in a mammal, comprising contacting or administering an effective amount of at least one IL-17 homolog polypeptide according to claims 13 with or to a cell, tissue, or organ of said mammal.

33. The method of claim 32, wherein said mammal is a primate.

34. The method of claim 33, wherein said primate is a monkey or a human.

35. The method of claim 32, wherein said effective amount is 0.001-10 mg/kilogram of said mammal.

36. A method for identifying compounds that bind at least one IL-17 homolog polypeptide, comprising;

admixing at least one isolated IL-17 homolog polypeptide according to claim 17 with at least one test compound or composition; and

detecting at least one binding interaction between said at least one IL-17 homolog polypeptide and the test compound or composition.

37. Any invention described herein.

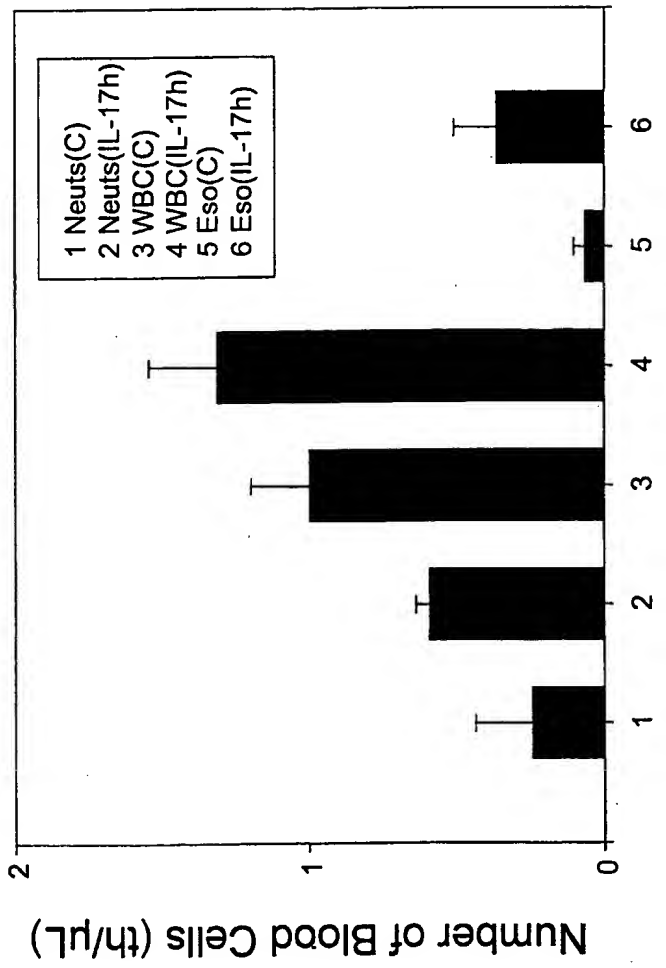


Figure 1

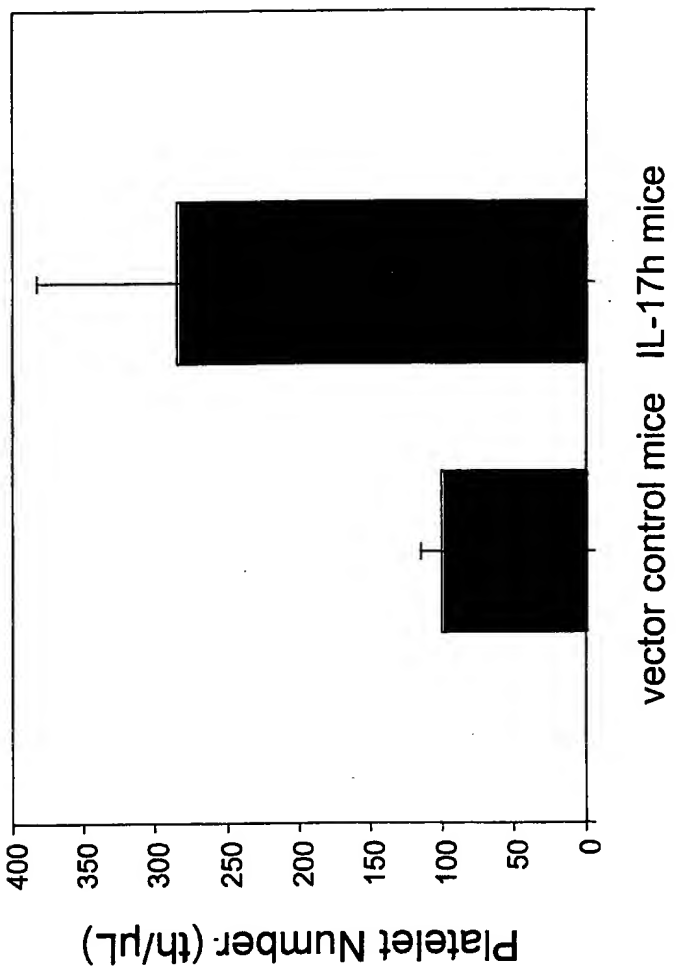


Figure 2

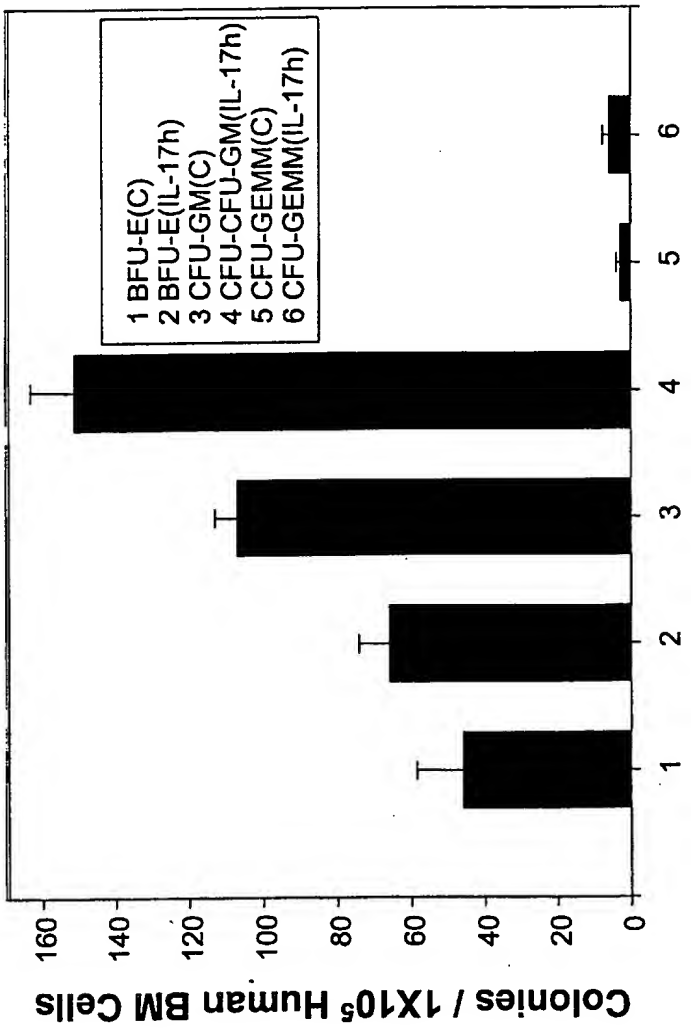


Figure 3

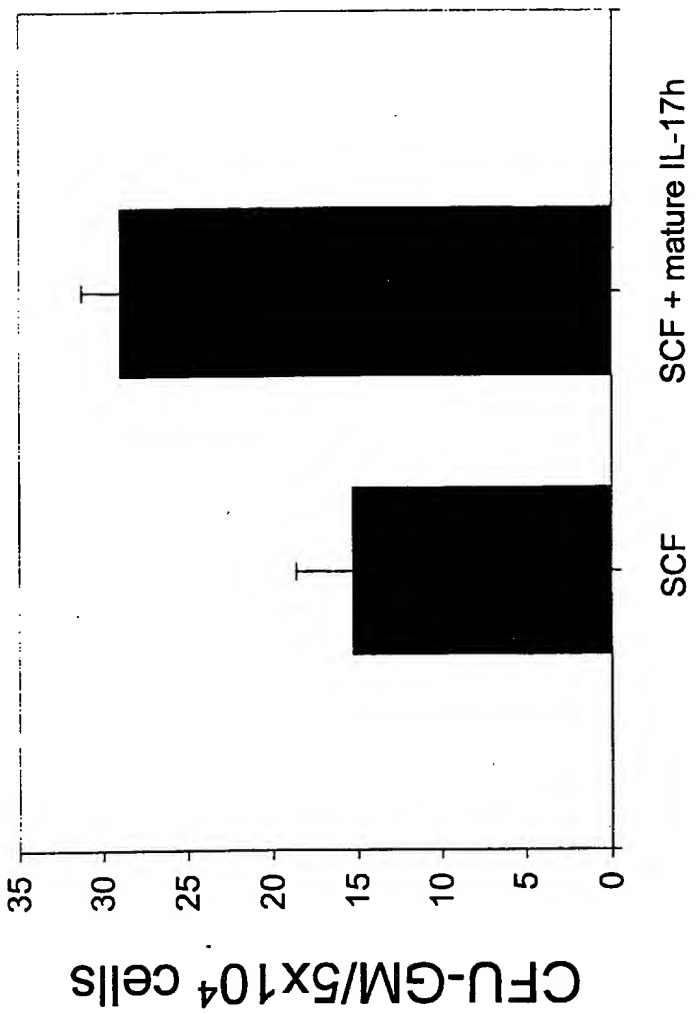
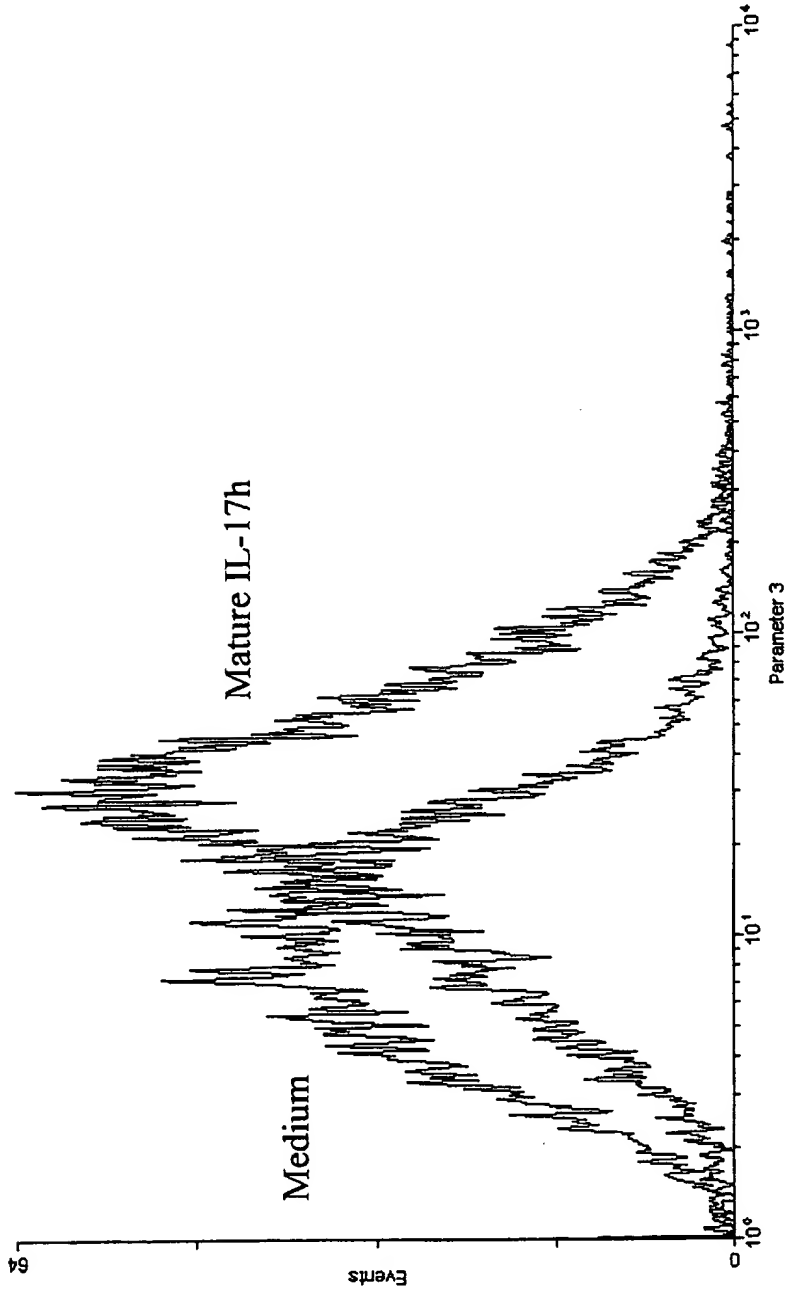


Figure 4

Figure 5

Effect of IL-17h on Human Dermal Fibroblast ICAM-1 Upregulation



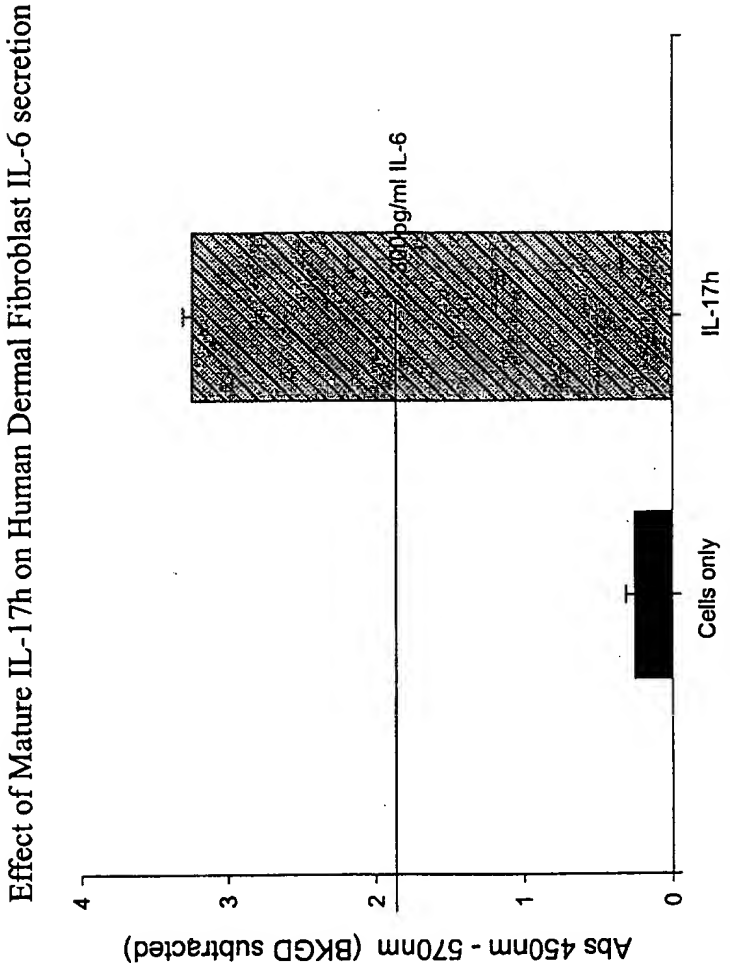


Figure 6

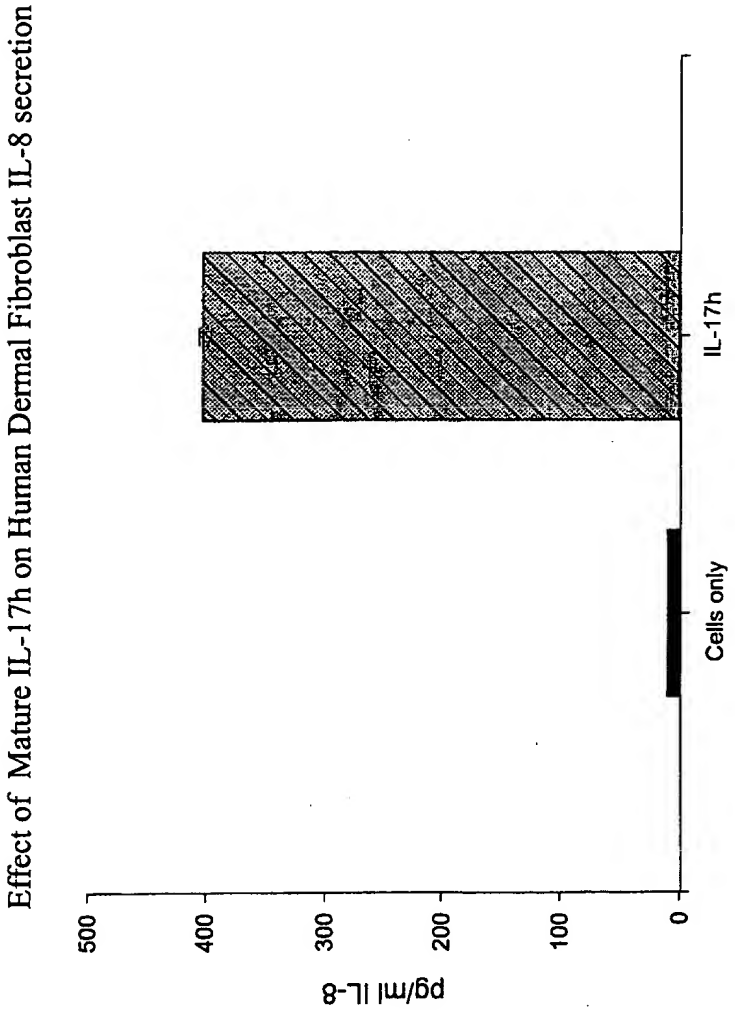


Figure 7

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 99/22678

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/19 C07K14/52 C12N5/10 C12Q1/68 C07K16/24
A61K38/19 A61K31/70 A01K67/027 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ROUVIER E ET AL: "CTLA-8, CLONED FROM A ACTIVATED T CELL, BEARING AU-RICH MESSENGER RNA INSTABILITY SEQUENCES, AND HOMOLOGOUS TO A HERPESVIRUS SAIMIRI GENE" JOURNAL OF IMMUNOLOGY,US,THE WILLIAMS AND WILKINS CO. BALTIMORE, vol. 150, no. 12, 15 June 1993 (1993-06-15), pages 5445-5456, XP002035505 ISSN: 0022-1767 cited in the application page 5453, left-hand column, paragraph 2 -right-hand column, paragraph 1 figure 3 abstract --- -/—	1,13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2000	02/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Cornec, N

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	YAO Z ET AL: "HUMAN IL-17: A NOVEL CYTOKINE DERIVED FROM T CELLS" JOURNAL OF IMMUNOLOGY,US,THE WILLIAMS AND WILKINS CO. BALTIMORE, vol. 155, no. 12, 15 December 1995 (1995-12-15), pages 5483-5486, XP000602481 ISSN: 0022-1767 cited in the application	
E	WO 99 60127 A (CHEN JIAN ;GENENTECH INC (US); LI HANZHONG (US); FILVAROFF ELLEN ()) 25 November 1999 (1999-11-25) the whole document especially sequences ID no.17,3,24,13,4 and 7 and the claims	1-22,24, 36

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/22678

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 27 (as far as it does not conern an in vitro method) and claims 28-35 are directed to a method of treatment of the human animal boddy (rule 39.1 IV PCT), the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 37

Claim 37 has not been searched because it does not have any meaning.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter onal Application No
PCT/US 99/22678

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9960127 A	25-11-1999	WO 9946281 A	16-09-1999

Form PCT/ISA/210 (patent family annex) (July 1992)